PB 179140

U.S.S.R. LITERATURE ON AIR POLLUTION AND RELATED OCCUPATIONAL DISEASES

Volume 15 :

A SURVEY
by
B. S. Levine, Ph. D.

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MAXIMUM PERMISSIBLE CONCENTRATIONS OF ATMOSPHERIC POLLUTANTS

Book 8

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"Meditsina" Press

Moscow, 1964

PB-179 140

Transliterated Russian Title
Predel'no Dopustimye Kentsentratsii Atmosfernykh Zagryaznenii
Vypusk VIII
Published by Meditsina 1964

The present English edition is a part of a survey conducted by
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of 3312 Northampton St., N. W.
Washington, D. C. 20015
awarded as Grant AP - 00176
By The National Air Pollution Control Administration

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FOREWORD

This is the eighth collection of papers prepared under the guidance of the Committee on Air Pollution of the USSR Public Health Inspectorate.

The collection contains papers approved by the Committee in 1962--1963. The first paper is a report presented by Prof. V. A. Ryazanov in 1962 before the International Symposium in Brussels: it is devoted to the determination of norms for atmospheric pollutants. Some of the articles (by V. A. Chizhikov, P. G. Tkachev, V. I. Filatova) present new experimental material for the determination of maximum permissible atmospheric air concentrations of waste products discharged into the air by new industries producing synthetic materials. The article by E. F. Kuperman on new synthetic material deals with chromium toxicity and its significance as an atmospheric pollutant. A. V. Mnatsakanyan describes new substances obtained ~~by her~~ in the course of investigating chloroprene. Some articles are devoted to the combined action of atmospheric pollutants (B. K. Baykov, V. K. Yefimova, Kh. Kh. Mannanova, K. A. Bushtueva).

This volume contains two articles by M. V. Alekseva discussing methods for the determination of atmospheric pollutants, and by V. A. Gofmekler outlining statistical methods for processing experimental data.

The appendix contains a revised and expanded list of maximum permissible concentrations and provisional method for computing dipersion of industrial wastes, such as ashes and sulfur dioxide, dischared into the atmosphere through their smoke stacks by electric power, heat and light stations.

M. S. Gol'dberg
V. A. Ryazanov

CRITERIA AND METHODS FOR THE DETERMINATION OF MAXIMUM PERMISSIBLE CONCENTRATIONS OF ATMOSPHERIC POLLUTANTS IN THE USSR

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the USSR Academy of Medical Sciences

V. A. Ryazanov

The fight against atmospheric pollution has become one of the urgent problems of the day; it has attracted the attention of scientists, politicians, and of the general world citizenry. Pertinent authoritative government institutions and enlightened citizen organizations demand that effective measures for the protection of air purity be made officially mandatory. However, it is first necessary to define pure air in practical terms; in other words, it is necessary to determine and legally adopt maximum permissible concentrations of atmospheric pollutants.

The concept of maximum allowable concentrations (MAC) of atmospheric air pollutants was advanced in the USSR. The Commission for the Determination of Maximum Permissible Concentrations of Atmospheric Pollutants was established in 1949 and the first list of MACs for air pollutants was approved by the Main USSR Public Health Inspection in 1952. At the time of this writing the Committee has established norms for forty atmospheric pollutants. The material dealing with the establishment of these norms was published in seven books entitled "Maximum Permissible Concentrations of Atmospheric Pollutants". Standards of atmospheric air purity are being investigated and are being formulated in Federal Republic of Germany, the USA and in many other countries. The time has come for the World Health Organization to call a conference for the expressed purpose of discussing the problems of establishing international maximum permissible concentrations of atmospheric pollutants, after representatives of different countries have presented their views and data for critical discussion.

Four different approaches to the determination of maximum permissible concentrations of atmospheric pollutants can be found in the literature. They can be summarized as follows:

1. Air composition can be considered normal only when it is in natural condition. According to this definition the procedure of determining maximum permissible concentrations is simple: Perform a sufficiently large number of air analyses away from populated areas and determine atmospheric air purity norms. This point of view cannot be accepted. There is no rational basis for the claim that any deviation from the so-called normal

atmospheric air composition exerts a detrimental effect on health. For every person there exists a threshold concentration below which no harm is done to the organism even under, so-called, chronic conditions.

2. For any given pollutant only that atmospheric concentration can be considered normal which cannot be practically removed with the available technological means. This position is completely unacceptable. Technically attainable concentrations can only fortuitously coincide with harmless concentrations. In most cases, they are higher than the harmless concentrations. Such norms can only hinder the search for better methods, since they sanction the techniques attained and do not stimulate progress.

3. Only those air pollutant concentrations can be considered normal which can be justitied economically, i. e. those consistent with the cost of their attainment, the cost being equal or less than the detriment to society. Such a purely commercial economical approach is unacceptable, since it contradicts the humane principles of medical science. The cost of human life and health are inestimable. In life insurance evaluations attempts are made to equate life and health with monetary compensations; yet, everybody understands the purpose for which this is done.

4. Only those concentrations of air pollutants can be assumed normal which do not exert on man, directly or indirectly, any harmful or unpleasant effects, do not affect his work capacity, and do not affect man's general state of being physically and emotionally. On this basis, concentrations of harmful substances are unacceptable if they affect adversely vegetation, the regional climate, atmospheric air transparency, and general living conditions of the population. This point of view has become the basis for the determination of maximum permissible concentrations in the USSR.

It does not necessarily follow from the above that in establishing legal norms considerations of economic and technical feasibility should not be taken into account. Hygienic norms of maximum permissible concentrations of atmospheric air pollutants must protect the population from adverse effects of such pollution. This cannot always universally be accomplished at or within a given time. Therefore, in addition to hygienic values of maximum permissible concentrations, sanitary norms can be adopted on a temporary and conditional basis. Lesser demands can be placed on the purity of atmospheric air for an agreed period of time, taking into account economic and technological conditions. Such norms can vary from one country to another, since they must take into account the economic conditions within each country. The temporary atmospheric pollution permitted by temporary norms must be replaced within a reasonable time by rationally and scientifically determined hygienic norms. This approach to hygienic norms of atmospheric purity stimulates technological and methodological progress in the fields of production and analysis.

In addition to hygienic and sanitary reasons for adopting norms of atmospheric pollutant concentrations there exist technological and economic reasons, namely, the discharge into the air and loss of valuable raw materials, intermediates, final products, etc. Such norms have nothing in common with hygienic norms, since, as stated above they are based on economic and technological considerations and can be used by public health agencies only as supplementary in exerting greater effect on industry in cases when the demands put upon it exceeded the hygienic and sanitary norms. Accordingly, there can be three types of norms limiting the discharge of harmful substances into the atmospheric air - sanitary-hygienic, technological, and economic.

The hygienic norms serve the interest of man from the biological aspect by aiming at the general improvement of the environment and living conditions. There can be no hygiene without sanitation. Therefore, sanitary norms are broadened hygienic norms by taking into account conditional factors, such as technological and economic feasibility. They may differ from country to country and from one period of economic and social development to another.

Technological norms are adopted to prevent the loss of valuable minerals and other substances into the atmospheric air to a degree compatible with economic considerations. The hygienic norms are of importance from the public health viewpoint. In determining maximum permissible concentrations, it is necessary to utilize first all available data pertaining to the effect of different poisons on the living organism. However, such data can serve only for preliminary orientation, since industrial toxicology deals with very high concentrations of poisons, not encountered in atmospheric air. In addition, environmental conditions of large urban dwellers and of industrial workers differ sharply. The main differences are as follows: Industrial workers are exposed to the action of harmful substances for 6 - 7 continuous hours, the general population is exposed to foul air 24 hours. Workers are generally physically the more resistant segment of the population. Among the urban dwellers there are old persons, small children, persons with different chronic diseases, and are usually more susceptible to the effects of harmful substances.

Maximum permissible concentrations adopted for indoor working premises are frequently arrived at by unrefined methods and are too high. This is especially true of maximum permissible concentrations adopted for harmful substances in the USA and in most West European countries. It is known that the maximum permissible concentrations of harmful substances adopted for air of working premises in the USSR are considerably lower than similar USA norms; yet, even the USSR MACs cannot be considered harmless for populated areas. Therefore, determination of maximum permissible concentrations in atmospheric air must be made by special experimental procedures using more than one species of laboratory animals. For this purpose experimental animals are kept in special chambers the air of which contained a known concentration of the substance under investigation. In the first stage of USSR investigations the animals are exposed to the poisoned air 2 hours daily for six months. In the second stage the animals are exposed to the poisoned air around the clock for 60 - 70 days. Exposure to the inhalation of air containing low concentration of the tested pollutant usually produces no marked changes in animals' behavior, body weight and elicits no statistically significant deviations in the animals' test indexes. At the same time, notable functional shifts do occur. In the following paragraphs the methods used and results obtained will be discussed.

Changes which take place in the higher nervous activity of the experimental animals under chronic experimental conditions are of high significance, since changes in cerebral cortex function take place early and at low concentrations, and since the cerebral cortex is highly sensitive to the effect of external environmental factors. The experimental animals first develop a dynamic stereotype followed by the development of toxicity. Experimental animals are observed daily or every other day and changes in behavior are noted. Changes in the higher nervous activity of rats are predominantly monotypical and are independent of the test substance used. The differences consist only in the magnitude of the threshold concentration. One of the early symptoms of the effect of different chemical substances on the higher nervous activity is the development of phase states. Differentiation disinhibition appears followed by the disappearance of individual reflexes, and finally the disappearance of all stereotype reflexes. With heavier intoxication, the natural conditioned reflex response to sight and smell of food also disappears.

In V. N. Kurnosov's experiments with mercury, concentrations of 2-5 $\mu g/m^3$ resulted in subsequent disappearance of the natural reflex to the sight and smell of food. It is important to note that these phenomena developed gradually, their severity increased with increasing duration of exposure. After cessation of exposure, the most severe symptoms disappeared first, and only then the less severe ones. The recovery period frequently lasted 30 days, and in some cases longer. In this manner the development of disturbances in higher nervous activity was of chronic type. The sensitivity of the conditioned reflex method was very high. Concentrations of mercury, lead, and benzene, considerably below the maximum permissible level for factories (indoor air), produced clear shifts in the higher nervous activity of rats.

The essence of developing changes in the higher nervous activity is in the gradual stimulability weakening and in the inhibition process, with subsequent development of defense inhibition. To evaluate the functional state of the cerebral cortex, changes in the chronaxies of antagonistic muscles are also studied. If the absolute chronaxy value depended on the central as well as the peripheral phenomena, and its changes cannot serve as a sensitive index of the state of the cerebral cortex, then the chronaxies ratio is determined by central effects.

Numerous investigators showed (Yu. G. Fel'dman, V. A. Gofmekler, Chao Chen-Sing, T. M. Shul'ga, V. I. Filatova, Li Cheng, R. Ubaydullayev, P. G. Tkachev, V. A. Chizhikov) that this test was very sensitive to the effects of toxic substances on the cerebral cortex under conditions of chronic experiments. Active concentrations were of the same magnitudes as the maximum permissible concentrations for factories (indoor air) or even lower (by a factor of 10 for aniline, by a factor of 30 for furfurol), etc.

The chronaxy of extensors is always higher than that of flexors in the control group: the curves of these chronaxies never tend to come closer throughout the entire observation period. In the experimental group flexor chronaxy gradually ascend to a level above the extensor chronaxy so that the two curves cross. After cessation of exposure the normal relationship of chronaxies is gradually reestablished. The change in the chronaxy ratio between the antagonistic-muscle chronaxies can be interpreted as the weakening of subordinate effects of the central nervous system. It can be assumed that this was conditioned by defense. Stimulation and inhibition processes of the organism are closely connected with the function of mediators. In this connection it is of interest to determine whether changes in cholinesterase activity may be sensitive indicators of functional shifts in the animal organism under conditions of chronic exposure to traces of atmospheric pollutants.

It was shown by many investigators (D. G. Odoshashvili, V. I. Filatova, P. G. Tkachev, R. Ubaydullayev, G. I. Solomin, V. A. Chizhikov) that changes in cholinesterase activity were produced by concentrations lower by a factor of 10-20 than the maximum permissible industrial concentrations. Thus, the given method can be regarded as sufficiently sensitive.

A very sensitive test employed in the USSR for the study of effects of atmospheric pollutants on the animal organism under conditions of chronic exposure is the determination of coproporphyrin in the urine. Coproporphyrin is determined spectrophotometrically. In animals subjected to chronic inhalation exposure, the course of coproporphyrin elimination with the urine differs from the control. Either increased or decreased elimination is observed. Carbon monoxide, butadiene, styrene, dimethylformamide, lower the rate of coproporphyrin elimination; lead and toluylene diisocyanate increase the amount of eliminated coproporphyrin. The concentrations of different toxic substances affecting coproporphyrin metabolism are considerably lower than their maximum permissible industrial concentrations (T. M. Shul'ga, Li Cheng, D. G. Odoshashvili, G. I. Solomin, V. A. Chizhikov, M. I. Gusev et al.)

The ratio of protein fractions in blood serum has been used recently as an indicator of the effect toxic substances had on the organism. Protein fraction analysis was made by paper electrophoresis with subsequent photoelectric densitometry of the phoregrams.

In experiments with chronic exposure to furfurol, R. Ubaydullayev showed that a concentration of 0.3 mg/m^3 was active enough to change the ratio between the finely dispersed proteins (albumins) and the coarsely dispersed proteins (globulins); the albumin content decreased, while the concentration of globulins increases, K. A. Bushtuyeva observed a similar effect in the action of low sulfur dioxide concentrations and in sulfuric acid aerosol. V. A. Chizhikov observed similar effects with low concentrations of toluylene diisocyanate, and P. G. Tkachev noted it in the action of low aniline concentrations. Changes in the plasma proteins dispersion, in spite of their lack of specificity, may indicate that the active factor disrupted the constancy of the organism's internal medium. This cannot be regarded as a normal phenomenon, particularly since the decrease in the dispersion of the serum proteins is not brief but rather prolonged.

Many other functional changes are also observed in the case of very low concentrations of toxic substances under chronic inhalation exposure conditions. These could be regarded as defense and adaptive reactions. They indicate that the external environment has deviated from the physiological optimum, thus forcing the organism to mobilize its protective and adaptive mechanisms. Even if the changes in the organism were not pathological in the true sense of the word, they indicated the unfavorable condition of the environment and decrease in the ability of the organism to adapt to the action of other factors requiring changes of the adaptive functions in the opposite sense. The need for such adjustment can arise as a result of sharp opposite environmental changes.

Two types of indicators of the degree of atmospheric pollution are used in the USSR: single and average 24 hour maximum permissible concentrations. Until now only 24 average MAC's had been considered. Their purpose is to prevent chronic effect of toxic substances through prolonged inhalation. Single maximum permissible concentrations are intended to prevent atmospheric air pollution capable of eliciting reflex reactions through irritation of respiratory receptors, primarily of the olfactory region of the nasal cavity. From the sanitary hygienic viewpoint outdoor air should have no extraneous odor unpleasant or undesirable to the inhabitants. Experience has shown that populations in general resent the presence in the ambient air of any type of extraneous odor and complain against atmospheric pollution with odoriferous substances of industrial waste or any other origin.

In the light of such considerations it is imperative that maximal allowable concentration for aromatic air contaminants be determined and set below the threshold of odor perception. This is usually done with the aid of volunteers whose sense of odor perception was normal and who were free of any nasal and olfactory pathology.

It is known that substances capable of stimulating the nasal cavity receptors and the upper respiratory tracts, in addition to the olfactory sensation or irritation, can also evoke reflexes such as changes in the frequency and amplitude of respiratory movements, spasticity of vocal cords and of the bronchial muscles, changes in the tonus of blood vessels, and changes in the functional state of the brain. Such reflex reactions have now become indexes in air pollution investigation. Changes in the rhythm and frequency of respiration can be of different forms. The frequency may increase, with a simultaneous decrease in the amplitude; the amplitude may become irregular. In most cases, changes in the respiratory movements appeared at pollutant concentrations sensed subjectively as odor and irritation of the nasal mucous membrance and of the throat. At concentrations below the level of subjective perception, no effect on the respiratory movements became manifest.

The method of optical chronaxy has received wide application in the USSR. After repeated preliminary optical chronaxy determinations the test subject is made to breathe a given concentration of a gas and the chronaxy is again determined. It was demonstrated that optical chronaxy changed under the effect of gas inhalation; it increased under the influence of odor emanating substances, as evidenced by the lowered stimulability of the central nervous system. According to I. P. Pavlov, stimulation of one area of the cerebral cortex can cause inhibition in other areas, in accordance with the principle of negative induction. With this as a basic principle experimental findings can be interpreted as indicating that stimulation originating in the olfactory area of the cerebral cortex produced inhibition in the optical area. Such inhibition is of reflex nature, as evidenced by its rapid disappearance on cessation of the olfactory irritation. It is interesting to note that with increasing gas concentrations the reaction also increased and sometimes became prolonged. The problem here consisted in determining the minimal concentration, i. e. the threshold of the given reaction. It was demonstrated that optical chronaxy changed only at concentrations equal to or higher than the olfactory threshold.

Data obtained by pneumography and optical chronaximetry showed that the olfactory threshold was an important hygienic parameter, since it characterized not only the feasibility of a subjective sensation of an inhaled substance, but also the appearance of reflex reactions. The latter may be in the form of changes in the external respiration or, more importantly, changes in the functional state of the cerebral cortex, among them cerebral vision areas. Inhibition of stimulability may be produced thereby. All this supports the need for forbidding the presence of small amounts of odor emanating substances in concentrations above the threshold of odor perception. The chronaximetric method proved insufficiently sensitive to detect below sensory reflex reactions of the olfactory receptors. Therefore, further studies were conducted with the aid of the method of dark adaptation.

An adaptometer makes possible to measure increases in sensitivity to light with the visual organ in the dark. The gas under investigation is fed in a given concentration to a cylinder from which the test subject breathes for 4½ min. (between the 15th and 20th min. of adaptation). After gas feeding has been discontinued the tester again breathes fresh air. Characteristic changes in light sensitivity appear during the 20th min. which vary depending on the concentration of the gas under investigation. When the concentrations are relatively high, a decrease in the sensitivity to light is observed. The higher the concentration, the more pronounced and prolonged is the decrease in sensitivity. For medium concentrations, testers clearly observed the double-phase character of the curve: initially sensitivity to light increased on the 20th min. after gas inhalation; it then sharply decreased on the 25th min.

In the case of low concentrations, an increase in the sensitivity to light was observed, which was not accompanied by a decrease below the norm. At still lower concentrations no significant changes in the dark adaptation were observed. Minimal concentrations for which statistically significant changes in the course of adaptation could be observed are regarded as threshold concentrations. The observed changes in light sensitivity were in good agreement with the observations of the famous Russian physiologists I. P. Pavlov and N. E. Vvedenskii; namely, weak stimuli stimulated, while strong stimuli inhibited the nervous system activity. After stimulation reached a certain intensity, depression inhibition set in.

Continued studies of dark adaptation disclosed that changes in sensitivity to light can be elicited by some substances in concentrations not perceptible by odor. The threshold of sulfur dioxide odor perception, for example, is 1.6 mg/m^3, etc. This fact is significant from the viewpoint of hygiene. It indicates that it is not always enough to designate as the MAC of an atmospheric pollutant on the basis of its odor nonperceptibility. The MAC in some instances must be below concentration of subconscious reflex thresholds, especially those capable of affecting the functional state of the cerebral cortex. In F. F. Erisman laboratory it was disclosed that not all substances were capable of eliciting subsensory changes in eye sensitivity to light. For example, formaldehyde, sulfuric acid, chlorine, and hydrogen chloride can change eye sensitivity to light only in odor perceptible concentrations.

It has been suggested that substances which affected primarily the olfactory nerve endings, elicited subsensory reactions, while substances with a trigeminal action, which affected the trifacial nerve were neutral in this respect. This suggestion requires thorough verification.

The electrocortical condition reflex test had also been applied to the determination of practical MAC of some air pollutants. This method has advantages over others, particularly over the adaptometric method. It is simple, requires little effort, and is highly sensitive. Persons with a pronounced alpha rhythm are selected for such studies. Observations are conducted in an electrically insulated and soundproof chamber. Each time a light signal is flashed to the test person, a temporary disruption of the alpha-rhythm synchronization is observed. The alpha-rhythm is reestablished at the end of the light signal. The test subject is given to breathe an odor emanating substance in low concentration so as not to cause alpha-rhythm desynchronization by itself. The test person inhales gas for 10 sec. followed by a light signal of 10-sec duration. The sequence is repeated several times (gas + light); it was observed that desynchronization began to appear prior to exposure to light, i. e. in response to the seemingly imperceptible odor. In this manner the imperceptible odor becomes the conditioned stimulus and elicits conditioned electrocortical reflex. The experiment is controlled entirely from outside.

The conditioned electrocortical reflex appears usually during the 6th and 8th light-gas combination, and is maintained up to the 20th combination, after which it gradually subsides. The experiment is then terminated. Experience shows that not all concentrations of an odor-emitting substance can evoke a conditioned electrocortical reflex. This reaction has its specific threshold. Repeated observations with different gas concentrations indicated a concentration which did not produce a reflex. This concentration is taken as one of the criteria in determining the maximum permissible concentration. The following are some experimental results: the perception threshold for sulfuric acid is 0.6 mg/m^3; the electrocortical reflex threshold is 0.4 mg/m^3; for sulfur dioxide the corresponding figures are 1.6 and 0.6 mg/m^3. For some substances the electrocortical reflex threshold is considerably lower than the perception threshold, for others the thresholds are identical. This leads to the hypothesis that olfactory active substances can evoke subsensory reactions, while trigeminally active substances cannot.

Recently a search has been made for more sensitive and convenient methods of investigating subsenory reactions to inhalation of odor emanating substances. Man's ability to assimilate an imposed rhythm has been used as an indicator of the functional state of the cerebral cortex. It is known that when a test person in an electroencephalographic chamber receives a light stimulus in the form of light flashes of a definite rhythm, the electroencephalogram will record vibrations corresponding to the frequency of the light pulses. The cortex assimilates the rhythm it imposed. Increasing the frequency of the stimuli increases the frequency of the biocurrents, while stronger light impulses raise the amplitude of the vibrations.

A. D. Semenko had shown that rhythm assimilation following the inhalation of a substance capable of affecting the olfactory analyzer differed from assimilation of the same rhythm following inhalation of pure air. The main difference is the fact that the amplitude of the assimilated rhythm decreased. Such impairment of the capacity of the central nervous system is produced by concentrations below the threshold of odor perception. Experience in applying the method of rhythm assimilation to the study of the effect of odor emanating substances on the state of the central nervous system has indicated that this method was as effective as the electrocortical conditioned reflex. It is felt that the potential inherent in the electroencephalographic method is significant; it is necessary to find other modifications of this method which might be even more sensitive and convenient under experimental conditions.

To sum up briefly, it should be noted that experimental methods are of great importance in determining maximum permissible concentrations of atmospheric air pollutants. The question in this connection arises as to why were not data on the incidence of disease among the population used for the same purpose.

The statistical method of analysis as applied to morbidity among populations living in sections of polluted atmospheric air and beyond, was used wherever conditions so indicated. Results showed that in all such investigations a higher incidence of disease prevailed among the segments of population who lived in densely polluted urban sections than among those who resided in the selected control sections. However, such data can not be used as a reliable basis for the assumption that the higher morbidity was due to atmospheric air pollution, since the effect of other active factors can not be excluded with absolute certainty. The most reliable data concerning morbidity as a result of atmospheric air pollution can be obtained among the infant population, especially where records of infant morbidity were numerically adequate and contained information on items essential to statistical analysis. For example, x-ray studies of chest cavities of children living near a large electrical power plant which discharged large amounts of ash containing up to 20% free silicon dioxide, showed hypersilicosis changes in the lungs (M. S. Gol'berg). The same observations were made by Rumanian investigators (Lupu and others). M. S. Sadilova examined children who lived near aluminum plants and found pronounced symptoms of fluorosis even though the water which the children drank contained only small amounts of fluorine. The changes mentioned could only be the result of fluoride dust and fluorine gas inhalation. M. E. Gusev found that children who lived near plants discharging large amounts of lead into the air eliminated via the urine statistically significant increased amount of coproporphyrine as compared with the controls. Eyzenbad found cases of berylliosis in persons who lived in the vicinity of beryllium-producing plants.

This shows beyond any doubt that atmospheric air pollution by toxic substances can induce in some residents pathologic changes characteristic of toxic air pollutants.

However, such data cannot be used unqualifiedly in the determination of maximum permissible concentrations of atmospheric pollutants, since they deal with concentrations known to be harmful and are entirely outside the sphere of harmless concentrations. In this connection it is well known that between the [illegible] of toxic and harmless concentrations there is a broad range of concentrations against which the organism develops adaptive and protective rather than pathological reactions. From the sanitary-hygienic viewpoint such concentrations cannot be considered permissible. The appearance of protective reactions should serve as a signal warning that the environment had deviated from the physiological optimum, i. e., from the hygienic norm. The Committee considers the appearance of protective and adaptive reactions elicited by the inhalation of atmospheric pollutants as indicating that the ambient air was in need of sanitary investigation and rectification. Maximum permissible concentrations of atmospheric air pollutants should not evoke such reactions. This is why population morbidity studies may show that the atmospheric air pollution may be excessively high without indicating the actual values of the maximum permissible concentrations. In this connection it should be stated that the problem of hygiene is not only the prevention of disease, but the securing of conditions favorable to the biological optimum.

BIOLOGICAL EFFECT AND HYGIENIC SIGNIFICANCE OF LOW TOLUYLENE DIISOCYANATE CONCENTRATIONS IN THE ATMOSPHERE

From the Department of Community Hygiene, The Central Institute of Post Graduate Mediciene

V. A. Chizhikov

In the last twenty years the new class of polymers, polyurethanes, has acquired wide industrial application, specifically as polyurethane foams, resins, lacquers, glues, coatings, elastomers, and fibers with polyurethane components. Polyurethanes are polymers in which the repeating link is a polyurethane group. The production of polyurethane plastics is based on the copolymerization of polyesters and isocyanates as the main components. The most widely used polyesters are those of adipic, sebacic, and phthalic acids in combination with glycols. Another component is the polyfunctional aromatic or aliphatic isocyanate, the most widely used of which is toluylene diisocyanate, a transparant, slightly yellow liquid with a sharp and unpleasant "amine" odor. Its density at 20°C is 1.22. It solidifies at 22°C to form colorless crystals. Its boiling point is 250°C. It dissolves without changing in hydrocarbons and halogenated hydrocarbons. Alcohols, amines, and other compounds containing active hydrogen react with it. Polyesters and diisocyanates react in a variety of modes, especially in the presence of water. Toluylene diisocyanate exists in the form of 2,4- and 2,6-isomers. The 2,4-isomer is somewhat more reactive than the 2,6-isomer, due to the different effects of the methyl group on the reactivity of the isocyanate groups in ortho- and para - positions.

Best industrial results in the preparation of foamed plastics are obtained with toluylene diisocyanate containing a mixture of 2,4- and 2,6-isomers in the ratios 87:13 and 83:17. In practice, however, standard mixtures of the above isomers in ratios 65:35 or 80:20 are used since such mixtures are prepared more easily. The 80:20 isomer mixture has a melting point of 14°C. The mixture most commonly used in the Soviet Union and other countries consists of a 65:35 ratio of 2,4- and 2,6-isomers. This mixture melts at approximately 8°C, enabling it to obtain foams at room temperature, which simplifies the process and improves the control of the reaction.

Throughout the experiments the present author used toluylene diisocayanate, b.p. 162°C (5mm), consisting of a 65:35 ratio of 2,4- and 2,6-isomers. Installations utilizing or manufacturing toluylene diisocyanate are potential sources of atmospheric pollution. Industrial methods of isocyanate synthesis are based on the reaction of phosghenes with amines and their salts (Hentschel, 1884). Similar to the majority of modern industrial chemical processes, production of toluylene diisocyanate is a continuous process. Industrial installations can become sources of atmospheric air pollution due to faulty conduit joints during equipment cleaning, industrial sampling, and some manual operations. High temperatures in industrial processes may produce significant concentrations of toluylene diisocyanate in the surrounding air.

Toluylene diisocyanate is used as raw material in the production of polyurethane foams, resins, glues, lacquers, coatings, fibers, and elastomers. Production of polyurethane foams in the USSR is a 3-component one-stage process. The production technology of elastic foams consists basically of the following operations: treatment of raw materials, preparation of the catalyst mixture, foaming and production of foam blocks, conveyor transport and cutting of blocks to given sizes, aging and block processing into desired items, and packing.

Toluylene diisocyanate can be emitted into the atmosphere at any point of the industrial production line, but it is most likely to occur in the beginning of raw materials unloading in mixing and pouring equipment, during belt conveing, and in the roll-trains where the exothermic polyurethane formation takes place.

Rigid and semi-rigid polyurethanes show considerable industrial promise, and even at this time are used widely in different branches of industry. Shortly after industrial production of polyurethane plastic began, many interesting communications were received concerning specific injuries suffered by workers handling diisocyanates, and specifically toluylene diisocyanate. Fuchs and Volade in 1951, first described the toxic symptoms appearing amoung workers handling toluylene diisocyanate. They reported that such workers showed irritation of mucous membranes of the upper respiratory tract, conjunctivitis, bronchitis, dry cough, shortness of breath, and oppressive chest tightness, expecially at night. In 1953 Reihl noted severe allergic reactions amoung workers employed in the production of toluylene diisocyanate. After the latent period, the affected individuals developed coughing and shortness of breath. Subsequently their general state of health deteriorated, symptoms of emphysema appeared followed by difficulty in exhaling, chronic bronchitis, secondary cardiac insufficiency and eosinophilia. Similar conditions were reported by Holmquist and Zundgren in Sweden.

In 1956 Schurman reported several cases of toluylene diisocyanate intoxication amoung workers which resulted in permanent disability. In 1957 Homa noted that illness set in when the concentration of toluylene diisocyanate in the air of industrial installations exceeded 0.21 mg/m^3. Johnstone in 1957, M. V. Gol'dblatt and Yu. Gol'dblatt in 1959, and others stressed the allergenic character of the illness caused by toluylene diisocyanate. Gapp conducted a large number of animal experiments in 1957. He noted a relatively weak reaction to toluylene diisocyanate introduced orally or applied to the skin; however, he pointed out its high toxicity upon inhalation.

Exposure of rats to 4.3mg/m^3 toluylene diisocyanate vapor proved lethal upon 6-hr exposure. Exposure of rates to the inhalation of toluylene diisocyanate vapor showed that 4.3 mg/m^3 inhaled for six hours proved absolutely lethal. Upon autopsy the mice showed pulmonary edema. Exposure of rats to air containing 10 mg/m^3 of toluylene diisocyanate for 6 hours daily over a period of 79 days failed to kill the mice. However, autopsies performed on some sacrificed rats showed the presence of bronchitis of different severity. The most pronounced toxic characteristic to toluylene diisocyanate appeared to be its allergenic property. As the concentration of the inhaled toluylene diisocyanate was reduced, its senitizing properties gradually abated.

The production of a synthetic foam rubber materials was initiated in the USSR comparatively recently, and all sanitary hygienic problems came into evidence later than in some foreign country. Some reports related to the results obtained with experimental animals exposed to the effect of toluylene diisocyanate were published in 1960 by I. N. Frolov and V. A. Tret'yakov of the Gor'kii Research Institute of Hygiene and Occupational Diseases. The authors found that acute and chronic poisoning of experimental animals appeared in 7-12 and 8mg/m^3 concentrations correspondingly. Local effects of the substance appeared in the form of conjunctival irritation and irritation of the mucosa of the upper respiratory passages; its absorptive effect appeared in the form of distrubed oxidation-reduction processes and as disturbed fatty and carbonhydrate metabolism, as pathomorphological changes in the form of granular liver and kidney distrophy and as fatty infiltration of the liver and muscles of the heart and other organs.

V. S. Filatova, M. S. Babosakina and T. V. Kurando presented their results in 1962 which indicated that the air of premises in which toluylene diisocyanate was producted contained 0.01 - 0.4 mg/m^3 of the substance vapor. Maximal concentration which exceeded the MAC occurred in premises where special operations in the production of synthetic foam rubber had been conducted. All workers complained of irritation caused by the end product including toluylene diisocyanate. The general complaints referred to burning eye sensation, lacrimation, ephiphora, nasal catarrh, etc. Most workers complained of headaches and excessive perspiration. Some complained of progressive loss of weight, insommia, coughing, dyspnea and abdominal pain. A study of the nervous system disclosed neurasthenic and vegetative effects. Most examined workers showed increased amount of reduced and total glutathione which pointed to a lowered oxidation-reduction activity in some workers; a study of the leucocytic formula showed a reduced number of neutrophiles, eosinophiles, and monocytes. These investigators were of the opinion that all mentioned disturbances, or all mentioned shifts were caused by the action of toluylene diisocyanate. The official MAC for toluylene diisocyanate in working premises was set in the USSR at 0.5 mg/m^3. Thus, according to the above cited results toluylene diisocyanate possessed strong toxic properties, and constituted a new industrial poison which appeared within the last two decades. It is expected that toluylene diisocyanate production will be gradually increasing in the USSR. Therefore, the toxicology of toluylene diisocyanate must be investigated thoroughly. Many authors are of the opinion that toluylene diisocyanate possessed allergenic properties, an opinion which has not been adequately substantiated. No evidence has been found in the literature which might indicate that low toluylene diisocyanate concentrations, such as are generally found in the air, might unfavorably affect human and animal organs. The same is true of toluylene diisocyanate concentrations generally found under industrial production conditions. Therefore, the present author had undertaken the task of determining in a more basic manner the effect of low toluylene diisocyanate concentrations on man and animal, and to determine its sanitary hygienic characteristics as an atmospheric air pollutant. The investigation began with a study of the acute toluylene diisocyanate effect on man in its concentration of threshold odor perception. Table 1 summarizes the results of studies made with thirteen toluylene diisocyanate concentrations amounting to a total of 395 determinations. Results indicated that 0.2 mg/m^3 of the substance constituted the minimal odor perception concentration, and 0.15 mg/m^3 the maximal odor nonperceptible concentration.

The effect of low toluylene diisocyanate concentration on brain electrical activity was investigated next. In this case use was made of a Kaiser 8-lead electroencephalograph following the procedures outlined for electroencephalographic studies by A. D. Semenenko, N. B. Imasheva, and V. I. Filatova in 1962. Encephalograms of the test subject were recorded simultaneously during stimulation by rhythmically flickering light and by encephalography. After several tests with fresh air alone for control purposes, tests were made with air containing different concentrations of the toluylene diisocyanate, as shown in Table 2. Results in that table show that inhalation of low toluylene diisocyanate concentrations for 5 minute intervals produced varying results on the electrical brain activity of different test individuals. Thus, test person "I" produced statistically reliable results only at 0.2 mg/m^3 concentration. The active concentration amounted to 0.15 mg/m^3 for two other test individuals, and for the remaining test individuals shifts which proved statistically significant were obtained with 0.10 mg/m^3 concentrations. No statistically reliable results were obtained with test persons inhaling 0.05 mg/m^3 of the toluylene diisocyanage. It should be noted at this point that changes in adaptation to the imposed rhythm were expressed as increased or decresed adaptation rhythms. The 0.2 mg/m^3

Table 1

DETERMINATION OF TOLUYLENEISOCYANATE PERCEPTION THRESHOLD

Number of observations	Concentration in mg/m³	
	Minimal perceptive	Maximal perceptive
2	0,20	0,15
3	0,30	0,20
3	0,35	0,30
5	0,45	0,35
5	0,60	0,45
2	1,00	0,60
1	2,60	2,00

Table 2

DETERMINATION OF TOLUYLENEISOCYANATE EFFECT ON BRAIN ACTIVITY

Number of observations	Concentration in mg/m³	
	Eliciting changes in the electroencephalogram	Eliciting no visible changes in the electroencephalogram
2	0,10	0,05
2	0,15	0,10
1	0,20	0,15

concentration depressed the electrical brain in all individuals, and at 0.01 mg/m³ elicited an increase in the total amplitude of the adopted rhythm in test individuals for whom such a concentration was inactive. Figure 1 presents adaptation rhythm curves of test individuals S and H. who inhaled fresh air and air containing different toluylene diisocyanate concentrations.

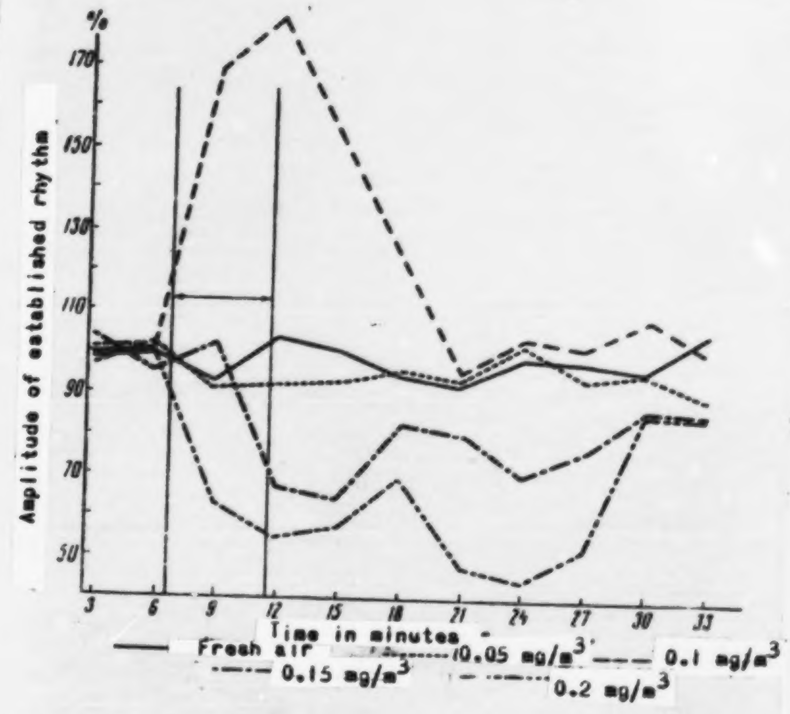

Fig. 1. Changes in acquired rhythm amplitude of female Sh. during inhalation of different toluyleneisocyanate concentrations

Arrow indicates period of inhalation of investigated concentration

Analysis of the curves shows that the 0.02 mg/m³ concentration more profoundly affected the electric brain conductivity than did the 0.15 mg/m³ concentration. Significant results were also obtained with inhaling air containing 0.10 mg/m³ of the toluylene diisocyanate. Such concentration of the substance augmented the electrical brain conductivity, as indicated by a considerable increase in the adopted rhythm amplitude.

Each test individual was given air to inhale containing four different test substance concentrations making a total of 100 experiments. All concentrations used were below the threshold of odor perception for the test individuals. Results of the experiment showed that 0.05 mg/m^3 of toluylene diisocyanate should be regarded as its maximal single concentration in atmospheric air. Determination of the maximal allowable daily average concentration was conducted by the method of chronic experiments, using white rats exposed to the inhalation of poisoned air 24 hours daily. In all, male rats were used which weighed 110 - 130 grams, and which were divided into four groups of 15 rats. Rats of group 1 inhaled air containing 2 mg/m^3, of the toluylene diisocyanate; rats of group 2 inhaled air containing 0.2 mg/m^3, and rats of group 3 inhaled air containing 0.02 mg/m^3 of the toluylene diisocyanate; rats of group 4 were used as controls. Concentrations of toluylene diisocyanate in the exposure chambers were checked three to four times daily in the first chamber, one to 2 times daily in the second chamber, and once daily in the third chamber. Average concentrations were 1.951 mg/m^3 ±0.223 in exposure chamber 1; 0.2036 mg/m^3 ±0.018 in chamber 2, and 0.0207 mg/m^3 ±0.0038 in chamber 3. Experimental animals were kept in the exposure chambers 84 days. Effect of different toluylene diisocyanate concentrations on the experimental animals had been observed and recorded in terms of changes in general behavior, general physical conditions, changes in their body weight, in the muscle-antagonists motor chronaxy changes, in blood cholinesterase activity, in blood serum protein fraction ratios, and in coproporphyrin eliminations with the urine.

Results of chronic toluylene diisocyanate tests indicated that the substance notably affected the general condition and course of body weight in animals of group 1. Animals of groups 2 and 3 gained weight at the same rate as did animals of the control group. They appeared well, were active and in every other way were indistinguishable from the control animals through the course of the experiment. Animals of group 1 which inhaled air containing 2 mg/m^3 of the poisonous vapor became restless early in the experiment; but this effect abated within a few days. However, soon they again became depressed. The nasal mucosa and eye conjunctiva showed signs of irritation, which persisted through the entire experimental period. However, only one of the test animals died as a result of the inhalation.

Experimental starvation clearly indicated that animals which inhaled air containing 2 mg/m^3 of the poisonous vapor lost their resistance. Curves presented in Figure 2 illustrate the effect of one day starvation on the weight of rats belonging to group 1. Muscle antagonists chronaxymetric determinations indicated that chronic inhalation of 2 mg/m^3, and also

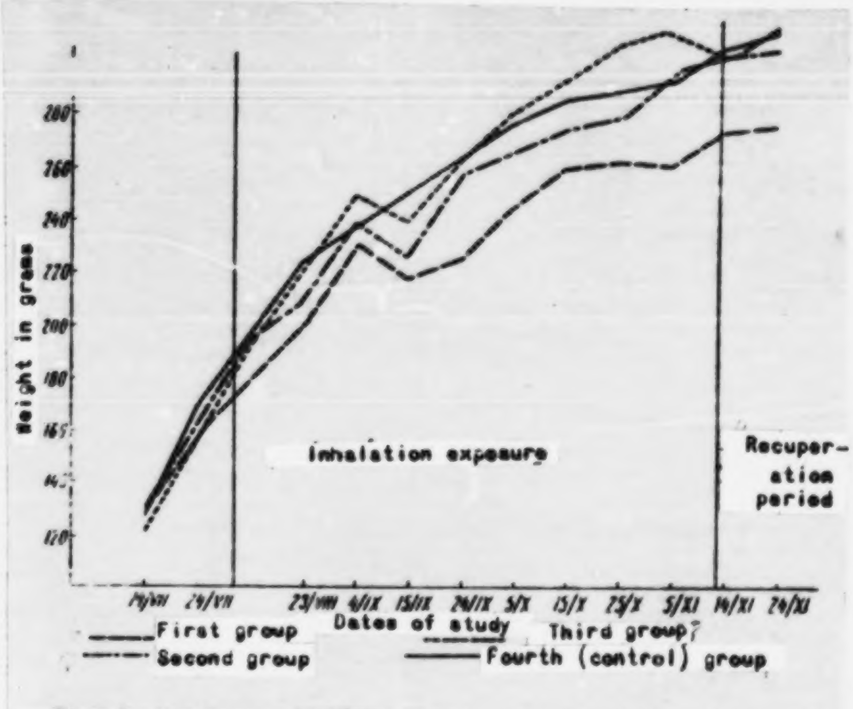

Fig. 2. Average weight changes in rats of different groups

(Note: See Figure 3 on page 19.)

0.2 mg/m^3, of toluylene diisocyanate concentration had a marked effect on the experimental animals' central nervous system. Thus, after one month's inhalation of the toxic vapor rats of group 1 developed reverse chronaxy ratios of the flexor and extensor muscles, which disappeared after the first month, and gradually returned to normal especially in rats of group 2, as shown by curves in Figure 3. Magnitudes of changes were checked and found statistically significant. No shifts in the chronaxy ratios were observed in animals belonging to group 3. The flexor and extensor muscle rheobase showed no substantial changes in any of the animals.

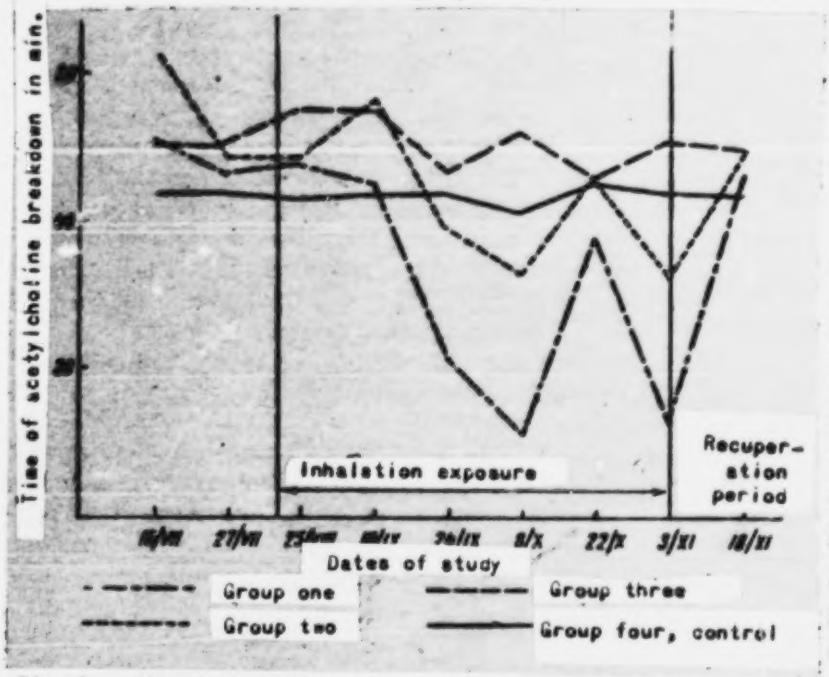

Fig. 4. Effect of toluylene diisocyanate on the activity of whole blood cholinesterase in rats of different groups.

Blood cholinesterase activity was determined by the A. P. Martynova modification of the A. A. Pokrovskii method. Results showed that enzyme activity increased by 35-40% in rats inhaling air containing 2 mg/m^3 of the poisonous vapor. Increase in cholinesterase activity was not as pronounced in rats belonging to group 2 which inhaled air containing 0.2 mg/m^3 of the toluylene diisocyante. Statistical reliability in either case amounted to 99%. During the recuperation period cholinesterase activity rapidly returned to its normal level, as shown by curves in Figure 4.

It has been known that rise in blood cholinesterase activity ran parallel to the degree of acetyl choline accumulation caused by profound arrest in

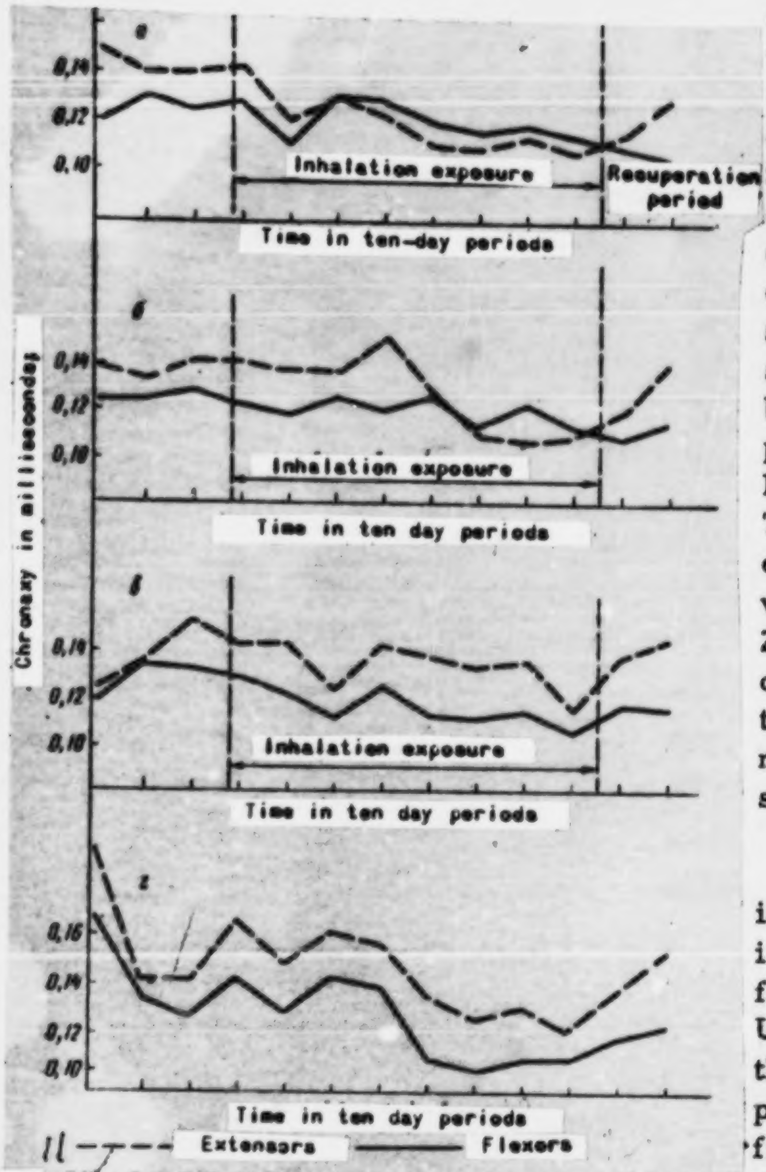

Fig. 3. Effect of toluylene diisocyanate on motor chronaxy on the different groups of experimental animals
a - group one; b - group two; c - group three - d - group four (control) group

central processes to a point at which one of the compensation mechanisms was disturbed functionally. Thus, results obtained in the investigation indicated that prolonged chronic inhalation of toluylene diisocyanate in 2 and 0.2 mg/m^3 elicited in the exposed animals significant shifts in the functional state of the nervous system paralled by the predominance of inhibition processes and enhanced acetylcholine accumulation in the tissues. This is evidenced by the fact that enhanced cholinesterase activity which occurred in rats of the first 2 groups was of a compensatory character. In 0.02 mg/m^3 concentrations, toluylene diisocyanate had no notable effect on the mediator system of the experimental animal.

Some authors also noted shifts in the process of protein metabolism in animals which received harmful substances chronically. R. Ubaidullaev reported in 1961 that the concentration of albumin dropped to lower levels, and globulin fractions rose to higher levels in the blood serum of animals chronically exposed to the effect of low furfural concentrations. G. V. Troitskii, V. I. Okulov and D. A. Sorkin established in 1961 that electrophoretic shifts in the blood serum protein affected by a variety of chemical reagents and monotypical characteristics expressed an increase in fractions which migrated at a rate characteristic of α_2 - and β_1 - globulins. N. F. Solodya demonstrated in 1960 that the normalization of protein fractions had been taking place less rapidly than did the normalization of the total protein compounds. Shifts in protein fractions apparently reflected not only new pathological changes, but to some extent shifts in the physiological levels. For instance, E. P. Smolichev found a rise in the globulin sections in persons as they moved from higher to lower altitudes. He also showed that under the stress of conditioned reflex activity the concentration of albumins fell to lower levels and of β- α- globulines rose to higher levels.

In the experiment conducted by the present author the effect of toluylene diisocyanate on blood serum protein fractions of rats was studied by the method of paper electrophoresis. Analysis of the foregrams was conducted by means of densitometer MGF, and the curves thus obtained had been integrated by the suspension method. Such tests were made with 5 rats of each group at 20 day intervals. No changes in the total protein were noted through the entire experimental period. Results were characterized by a degree of preliminery dispersion. For this reason they were analyzed as follows: Averages of all determinations were used as controls, and all successive shifts in the protein fraction levels which had occurred during the experimental period had been evaluated with reference to such averages. Data presented in Table 3 indicated that first to undergo significant changes in levels were the albumin fractions, followed by the β- globulin fractions; α- globulin fractions showed no significant shifts from the original levels. Thus, it can be seen that inhalation of toluylene diisocyanate caused a drop in the level of albumin fractions and a parallel rise in the concentration of α- and γ- globulins. The statistical significance of the shifts were more pronounced in rats of group 1, the statistical reliability of which amounted to 95%. Rats of group 3 and of the control group manifested no statistically significant changes in their blood serum protein elements. The investigated shifts reached their maximum at the end of the exposure period and persisted through the entire recuperation period.

Table 3

PERCENT OF CHANGES IN BLOOD SERUM PROTEIN FRACTIONS OF RATS SUBJECTED TO CHRONIC POISONING WITH TOLUYLENE DIISOCYANATE

Protein fraction	Observation period	Animal group			
		First	Second	Third	Control
Albumins	Before inhalation	40,83	40,83	40,83	40,83
	During inhalation	32,11	36,69	37,14	39,19
	Recovery period	34,75	39,25	38,38	38,40
α-globulins	Before inhalation	18,45	18,45	18,45	18,45
	During inhalation	21,16	17,46	18,84	19,67
	Recovery period	20,30	21,80	18,11	18,16
β-globulins	Before inhalation	24,81	24,81	24,81	24,81
	During inhalation	29,11	27,47.	26,22	26,00
	Recovery period	27,97	24,65	26,00	27,80
γ-globulins	Before inhalation	16,10	16,10	16,10	16,10
	During inhalation	18,00	18,41	17,77	15,00
	Recovery period	16,95	14,28	17,45	15,60

As previously mentioned, parallel with the blood serum protein, study determinations were made of the rates of coproporphyrin elimination with the urine. The effect of chronic administration of low concentrations of harmful substances on porphyrin metabolism had not been adequately investigated. However, it is known that porphyrin substances played an active role in the general metabolism, and it is assumed that even temporary blocking or change in the direction of their conversion can serve as an index of abnormal changes in the course of normal general metabolism. M. I. Gusev, G. I. Solomin, Lee Shean, and others had shown that chronic administration of harmful substances in low concentrations had a substantial effect on the dynamics of coproporphyrin elimination with the urine. Urine specimens had been collected from five rats each group of rats which were kept in specially equipped exposure chambers. Porphyrin was extracted from the urine by the Fisher method. Quantitative and qualitative determinations of coporprophyrin were made with the aid of a spectrophotometer SF-5 in the range of waves 400 - 410. Rats of group 1 eliminated coproporphyrin with their urine at a higher level beginning with the 4th week of the toxic vapor inhalation. This increased level of urinary coproporphyrin persisted to the end of the recuperating period. Lower level changes, but still statistically amounting to 95% reliability in the elimination of urinary coproporphyrin, were noted in rats of group 2. Following the termination of the toxic vapor inhalation levels of coproporphyrin eliminated by the rats with their urine rapidly returned to normal. Rats of group 3 showed no statistically significant changes in the levels of coproporphyrin elimination with the urine, as is illustrated by curves in Figure 5.

Thus, persistent chronic poisoning of white rats with toluylene diisocyanate for 84 days indicated that 2 and 0.2 mg/m^3 of the vapor unmistakably affected the experimental animals, as shown by a drop in the rate of body weight gain and an increase in the level of cholinesterase activity, in a change of the motor chronaxy ratios, in the character of prophyrin metabolism, and in the blood serum protein fraction ratios.

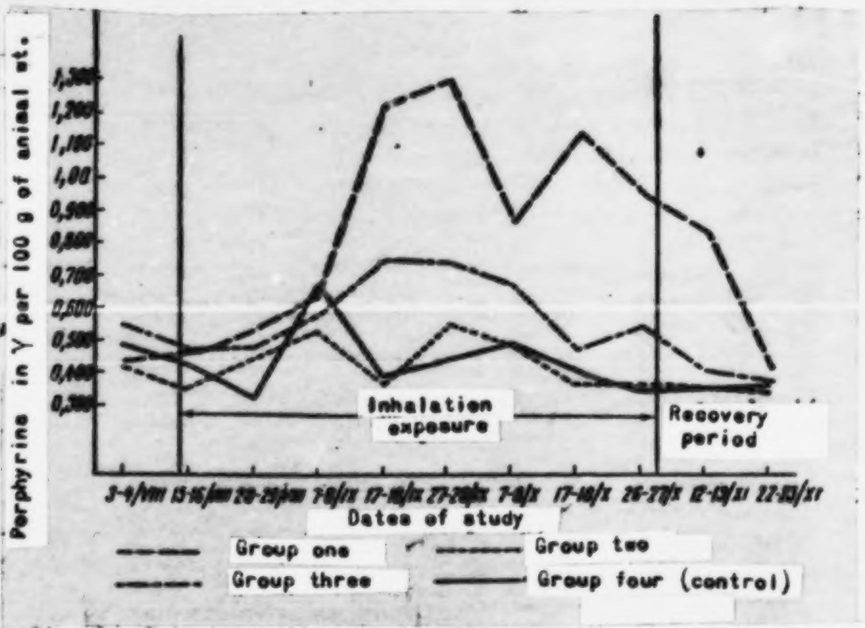

Fig. 5. Toluylene diisocyanate effect on rate of urinary coproporphyrin elimination by the experimental animals of each group

In 0.02 mg/m^3 concentration to toluylene diisocyanate elicited no changes in the experimental rats through the entire course of exposure; for this reason 0.02 mg/m^3 of the substance can be regarded as the 24 hour average concentration for this substance in atmospheric air.

During June and September of 1962, 107 single air samples had been collected in the proximity of a plant where toluylene diisocyanate was used as a raw product. The plant had air purifying installations which, however, were not in operation, so that all harmful discharges into the atmosphere represented the raw unpurified air pollutants produced by this plant. The plant operated in 2 shifts in a way that the final product was produced during the first shift, and the preparatory operations were conducted during the second shift. Data presented in Table 4 show that toluylene diisocyanate concentrations in the atmospheric air rapidly fell to lower levels as the distance from the pollution source increased, and at 300 m from the pollution source the concentration did not exceed the proposed maximal limits for allowable single and 24-hour average concentrations. It should be noted that a slight change in the production technology such, for instance, as the simultaneous operation of the first and second production stages, or an increase in the rate of total production, might lead to a substantital increase of the pollutant concentrations in the atmospheric air.

Table 4

INTENSITY OF ATMOSPHERIC AIR POLLUTION WITH TOLUYLENE DIISOCYANATE IN THE PRODUCTION PLANT ENVIRONMENT

Distance from pollution source	No. of collected and tested air samples		Minimal concentration in mg/m^3	Concn. distribution in samples in mg/m^3		
	Total	No. exceeding test sensitivity		Above 0.05	0.05—0.02	Less than 0.02
75	25	18	0,075	6	10	2
200	50	22	0,024	—	4	18
300	32	1	0,006	—	—	1

Conclusions

1. The threshold of toluylene diisocyanate odor perception for most odor sensitive persons was established at 0.2 mg/m^3; 0.15 mg/m^3 was the maximal nonodor-perceptible concentration of toluylene diisocyanate vapor.

2. The threshold effect of toluylene diisocyanate on electric brain activity was established at 0.10 mg/m^3; 0.05 mg/m^3 of the vapor elicited no changes in the electrical brain activity.

3. It is proposed that 0.05 mg/m^3 of toluylene diisocyanate be adopted as the maximal single limit of allowable concentration in the atmospheric air.

4. Exposure of white rats to the inhalation of air containing 2 and 0.2 mg/m^3 of toluylene diisocyanate 24 hours a day for 84 days elicited in the experimental rats an arrest in their weight gain, enhanced cholinesterase activity, affected their motor chronaxy, elicited a change in the protein fraction ratios, and affected porphyrin metabolism. No changes in any of the above mentioned phases could be detected in white rats exposed to the chronic inhalation of 0.02 mg/m^3 of toluylene diisocyanate.

5. It is proposed that the average 24 hour limit of allowable toluylene diisocyanate concentration in the atmospheric air be set at the level of 0.02 mg/m^3.

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HYGIENIC ANILINE CHARACTERISTICS AS A POLLUTANT OF ATMOSPHERIC AIR

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Aniline - C_6, H_5, NH_2 - known as "aromatic amine", is a substance widely used in the aniline dye industry, in the production of plastic materials, synthetic rubber, etc.; it is also an intermediate product in the synthesis of drugs and in the production of naphthaline intermediates. Aniline toxic properties have been investigated by intragastric adminstration, external skin application, in comparatively large doses. The mechanism of aniline poisoning has been explained differently by different authors. Some believe that aniline attacks basically the nervous system. Others believe that it had a destructive effect on the blood, while still others believe that it had a destructive effect on the liver. Accordingly, different claims have been made by different authors regarding the basic symptoms of aniline poisoning. The dose and the time which bring into evidence a given type of clincal aniline poisoning picture depend upon the route of its entrance into the organism. Effect of low aniline concentrations chronically inhaled by humans has not been adequately investigated and described in the literature. Except for the work of M. K. Grozdovskii on the effect of aniline vapor present in the air surrounding an aniline plant practically nothing has been described in the literature concerning its significance as an atmospheric air pollutant. Therefore, the present author attempted to determine experimentally the MAC of aniline in the atmospheric air of inhabited areas.

The threshold of aniline odor perception was investigated with the cooperation of 17 normal volunteers with whom 484 tests have been made. Results indicated that such a threshold ranged between 6.37 and 0.25 mg/m^3. Many of the volunteers have been tested 19 to 40 times and only one had been tested 11 times. Data presented in Table 1 indicates that for most odor sensitive persons the threshold of aniline vapor concentrations was at 0.37 mg/m^3.

The effect of aniline vapor on the reflex activity was tested by the method of functional electroencephalography using the amplitude of acquired α-rhythm in response to blinking light stimulation. In this case 6 volunteers of normal odor perception and clearly defined α- rhythm, have been used as test subjects; each of them had undergone an average of 14 observations of aniline effect in 0.3, 0.15, and 0.07 mg/m^3 vapor concentrations in the air, and of pure air for control purposes. Results are shown in Table 2. Statistically significant shifts in the direction of reduced amplitude of the adjusted α-rhythm at the 0.3 mg/m^3 aniline vapor concentration were obtained with all six test volunteers, and in only three in the case of 0.15 mg/m^3 concentrations. In the case of test person M. V., whose threshold odor perception was at 34 mg/m^3, changes were discerned only during the first minute of the recuperating period following the inhalation of air containing 0.07 mg/m^3 of the aniline vapor. Therefore, this concentration was regarded by the present author as the minimal active concentration which stimulated the brain cortex processes. Results of the investigation led the present author to assume 0.05 mg/m^3 of aniline as the maximal single allowable concentration. The average 24 hour concentration of aniline in the atmospheric air was established by experiments conducted over a period of 80 days using 80 male rats weighing 90 to 150 grams, which inhaled aniline vapor 24 hours a day. The 80 rats were divided into four groups of 20 specimens each. Rats of group 1 were exposed to aniline vapor inhalation in 3 mg/m^3 concentrations, which is also the limit of allowable aniline vapor concentration for the air of production premises. Rats of group 2 were exposed to the inhalation of air containing 0.3mg/m^3. of the aniline vapor, which is close to the concentration of odor threshold perception; rats of group 3 were exposed to the inhalation of air containing 0.03 mg/m^3 of the aniline vapor, or a concentration below the minimal which affected the human brain cortex. Rats of group 4 served as controls. The same indexes as were described in the preceding investigation were used as determining factors of aniline vapor effect on the living organism. For obvious reasons formation of methemoglobin and histologic changes had also been taken into consideration. All investigations had been initiated about thirty days before experimental inhalation of the aniline vapors, and were continued through a 20 day recovery period, making a total of 130 days. Constancy of aniline vapor concentration in the air in the inhalation chamber was checked as described in previous experiments.

Throughout the inhalation periods rats of all the groups

Table I

RESULTS OF ANILINE THRESHOLD ODOR PERCEPTION DETERMINATIONS

No. of tests	Initials of tester	Minimal perceptible concn. in mg/m^3	Maximal perceptible concns.	Total observations
1	F.D.	2,82	2,78	25
2	S.L.	2,82	2,78	19
3	T.P.	2,21	2,07	33
4	S.G.	2,21	2,07	30
5	Kh.Z.	1,74	1,68	30
6	B.K.	1,74	1,68	26
7	L.Sh.	1,74	1,68	22
8	D.A.	1,74	1,68	21
9	P.B.	1,74	1,68	28
10	P.A.	1,74	1,68	23
11	I.N.	0,88	0,74	35
12	Kh.Yu.	0,88	0,74	38
13	T.K.	0,88	0,74	28
14	M.Kh.	0,88	0,74	11
15	Kh.V.	0,88	0,74	36
16	M.V.	0,37	0,34	40
17	I.N.	0,37	0,34	39

appeared healthy, active and gained body weight at a relatively normal rate. No statistically significant changes in the weight of rats of all the three groups had been observed. Determinations of rheobase and muscle antagonists chronaxy were made with five rats of each group. Such determinations were made under identical conditions at 10 day intervals. Data related to the muscle antagonists chronaxy ratios are illustrated as curves in Figure 1. Reverse chronaxy ratios appeared in all 5 rats of group 1 which inhaled air containing 3 mg/m^3 of the aniline vapor on the third decade of the vapor inhalation. The chronaxy ratio gradually returned to normal during the first ten days of the recovery period. In four rats of group 2, which inhaled air containing 0.3 mg/m^3 of the aniline vapor the reverse chronaxy ratio appeared during the fifth - seventh exposure decade, the intensity of which was considerably greater than in rats of group 1. The chronaxy ratio in rats belonging to group 3, which inhaled air containg 0.03 mg/m^3 of the aniline vapor, remained practically the same as it was in rats of control group 4, through the entire period of the investigation.

Changes in cholinesterase activity were determined by the A. P. Martynova modification of the A. A. Pokrovskii method, using five rats from each group. Determinations were made at 14 day intervals. Curves in Figure 2, show that the statistically reliabile cholinesterase activity increase was elicited by the inhalation of air containing 3 mg/m^3 of aniline vapor. Results obtained by the present author were in agreement with results obtained by V. K. Navrotsii, V. I. Lukashev, and others, which indicated that light chronic poisoning of rabbits with 0.15 g/kg aniline doses administered subcutaneously brought about a two-fold rise in the rate of cholinesterase activity.

G. N. Vas'ko and K. A. Lavrovskii noted that aniline vapor produced porphyrinuria. L. G. Chernysheva studied porphyrin metabolism in different stages with different diseases which included aniline poisoning.

She found that porphyrinuria rose to higher levels, and regarded the phenomenon as the organism's compensatory reaction to profoundly disturbed metabolic processes. N. I. Gusev and Yu. K. Smirnov, G. I. Solomin, and others studied the rate of coproporphyrin elimination with the urine following experimental inhalation of air containing low concentrations of some harmful substances. Aniline acted as a tissue respiration and oxidative enzyme depressant which gave rise to hemoporphyrin complexes. Therefore, the present author studied the course of urinary coproporphyrin elimination by the Fisher method based on the extraction of the substance with other followed by spectral photometric determinations. During the preliminary period of the investigation and also during the recuperation period, the coproporphyrin elimination rate in rats of groups 2 and 3 varied within the limits of 0.39 - 0.93 μ per 100 g of the animal body weight. Rate of coproporphyrin elimination via the urine rose to 1.11μ per 100 g of animal body weight beginning with the 8 day of inhalation and persisted through the entire experimental period, which exceeded the rate of urinary coproporphyrin elimination in rats of all other groups. Rate of coproporphyrin elimination in the course of aniline vapor inhalation by the different groups was as follows: rats of group 1 which inhaled air containing 3 mg/m^3 of aniline, the elimination rate was 0.78 ± 0.19 μ; in rats of group 2 which inhaled air containing 0.3 mg/m^3 of aniline vapor, the elimination rate was 0.58 ±15 μ; in rats of group 3, which inhaled air containing 0.03 mg/m^3 of aniline vapor, the elimination rate was 0.48 ± 0.19μ; rats of the control group eliminated coproporphyrin at the rate of 0.48 ± 0.10 μ. On the basis of such results the present author believed that inhalation of air containing 3 mg/m± of aniline vapor elicited in the experimental animals a moderate coproporphyrinmuria. Statistical analysis verified the validity of the experimental results.

Aniline easily combined with blood proteins forming methemoglobin and Heinz bodies; therefore, the present author used the electrophoretic method of blood serum protein analysis, results of which are presented in Table 3. According to the data, adminstration of air containing aniline in the indicated concentration elicited, no statistically reliable changes in the total blood serum protein concentrations. On the 10th day of experimental inhalation of air containing 3mg/m^3 of the aniline vapor the concentration of albumin in the blood serum of the rats showed statistically verifiable changes. Globulin

Table 2

ANILINE EFFECT ON SUMMARY AMPLITUDE OF ACQUIRED RHYTHM

Initials of test person	Analine concns in mg/m^3		
	0.30	0.15	0.07
K.Ea.	+	—	—
B.E.	+	—	—
G.D.	+	—	—
M.G.	+	+	—
B.N.	+	+	—
M.V.	+	+	+

concentrations showed similar changes on the 30th day of experimental inhalation expressed in the form of reduction in the albumins and increase in the α- and β- globulins. Concentrations of globulins persisted at higher fluctuating levels to the end of the experimental inhalation period; the α-globulins failed to return to the original level even on the 22nd day of the recovery period. High changes in the blood protein fractions were noted in rats No. 43 of group 1 in which the albumin concentration dropped from the original 39.7% to 15.5% on the 10th inhalation day and the α- globulins rose from the original 19.2 to 26.9% while the β- globulins rose from the original 26.9 to 37.3%. Changes in albumin and globulin concentrations of rats belonging to group 2 were of no great significance, while blood concentrations of these elements in rats of group 3 were comparable to those found in rats of the control group. Zarone and Scala administered 1.5 g of aniline to rabbits subcutaneously, following which they analyzed the blood plasma electrophoretically; they could detect no significant changes in the blood protein picture; however, they noted a significant increase in the concentration of lipoproteins.

The present author is of the opinion that above reported shifts in blood serum protein fractions reflected disturbed liver synthesizing function brought about by the inhalation of air containing 3 mg/m^3 of aniline vapor.

The methemoglobin forming property of aniline has been known for some time. In this connection the present author conducted quantitative methemoglobin determinations in the blood of five rats of each group at 20 day intervals using the Horn method. Blocks in Figure 3 show that methemoglobin in rats of group 1 amounted to 4.86% on the 15th day of experimental aniline vapor inhalation. On the 35th day the methemoglobin concentration rose to 8.06%, and in rat No. 63 of this group, the methemoglobin concentration was as high as 13.9%, which gradually receded to the end of the experimental inhalation period. Methemoglobin concentration in the blood of rats belonging to group 2, amounted to 2.64% on the 35th day of the experimental aniline vapor inhalation and to 3.82% on the 35th day of the aniline vapor inhalation. It then dropped to and persisted at 1.39% to the end of the experiment. The methemoglobin concentration in the blood of rats of group 3 and of the control group was below the level of the test sensitivity. The present author came to believe that rise in the blood methemoglobin concentration in rats inhaling aniline vapor followed by their drop to the level of nondetectable concentration, even in the face of continued aniline inhalation, could be explained as follows: It is known that the process of methemoglobin formation was reversible; therefore, formed methemoglobin could convert to free hemoglobin in the course of time. This physiological process operated continuously under normal physiological conditions depending upon the concentration of reducing substances present in the blood. The animal organism possessed a degree of adaptability to oxygen deficiency created by the formation of methemoglobin in the course of aniline vapor inhalation.

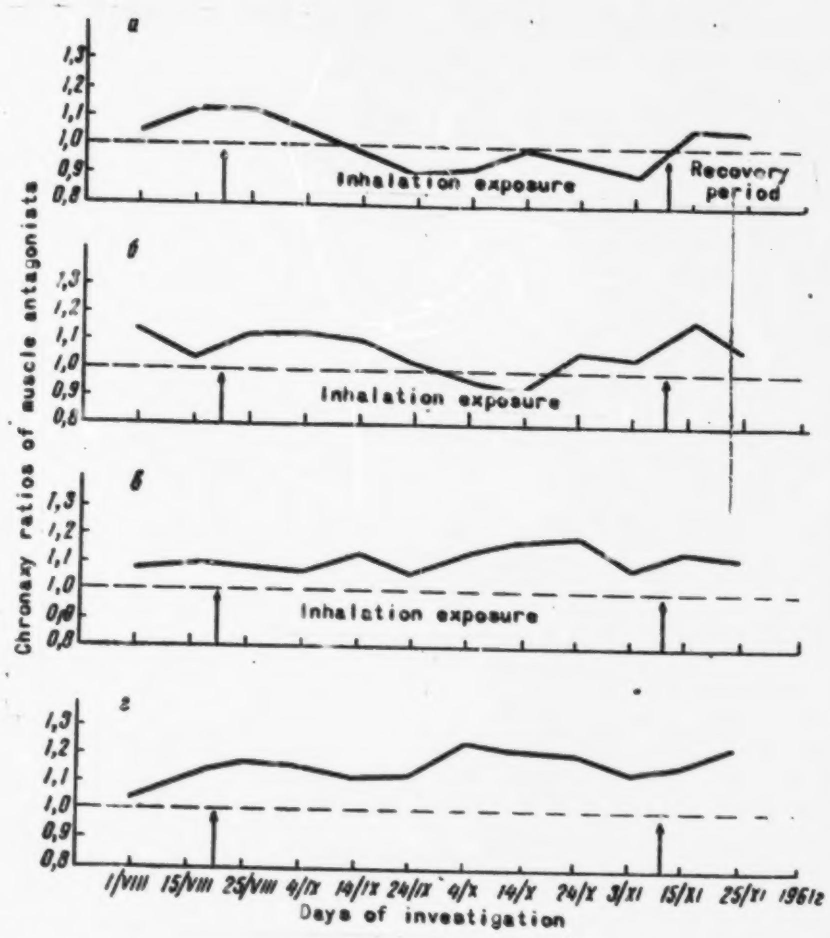

Fig. 1. Muscle antagonists chronaxy ratio in rats exposed to the effect of different aniline vapor concentrations
a – Group 1; b – Group 2; c – Group 3; d – Group 4 (control).
Solid line indicates ratios on extensors to flexors; dotted line indicates equal levels of both chronaxy types.

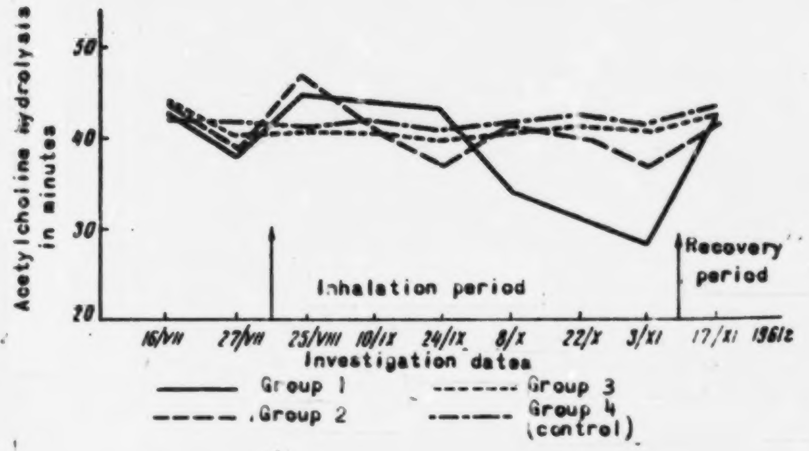

Fig. 2. Rats' whole blood cholinesterase activity during aniline vapor inhalation

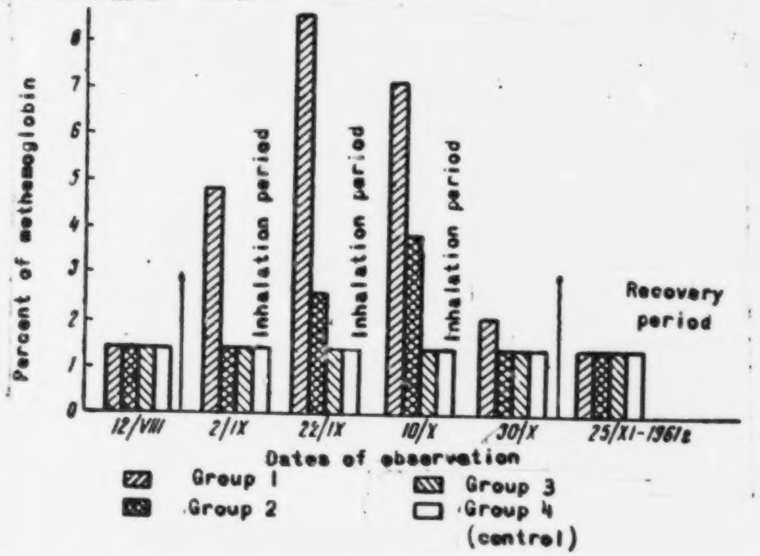

Fig. 3. Amount of blood methemoglobin in rats during period of aniline vapor inhalation

At the same time, through the process of compensatory reaction, the number of reducing elements increased in the blood, which leads to a rapid reconversion of the methemoglobin to hemoglobin.

Some authors are of the opinion that the number of Heinz bodies ran parallel to the amount of formed methemoglobin, and to the duration of methemoglobinemia produced by aniline vapor inhalation. S. M. Henkin and E. Kantorovich did not agree with this opinion, since they found Heinz bodies in blood free from methemoglobin. However, evidence found by the present author indicated that Heinz bodies were found in the blood of persons who inhaled air containing high aniline vapor concentrations.

A study was made of the formed elements in the blood of 5 rats of each group. A statistically verifiable drop in the number of erthrocytes was found in rats of group 2, as illustrated by curves presented in Figure 6. The course of erthrocyte reduction can be presented for convenience in three stages. In the first stage, which included the first 13 days of aniline vapor inhalation by the experimental rats, the number of erthrocytes rose from 6,500,000 to 7,900,000; in the second stage erthrocytes in the blood of rats belonging to groups 1 and 2 dropped correspondingly from 7,700,000 to 6,700,000 and from 7,900,000 to 7,400,000. At the same time erthrocytes in the blood of rats belonging to groups 3 and 4 rose only slightly.

Table 3

BLOOD SERUM PROTEIN FRACTIONS IN RATS INHALING ANILINE VAPOR IN PERCENT

Animal groups	Background					Inhalation period					Recovery period				
	Total protein	Albumin	Globulins			Total protein	Albumin	Globulins			Total protein	Albumin	Globulins		
			α	β	γ			α	β	γ			α	β	γ
First	8,15	46,4	19,2	21,3	13	7,92	31,3	23,7	30,5	14,1	7,53	34,4	27,7	26,9	11,3
Second	7,88	43,3	18,4	24,4	13,8	8,32	38,6	18,5	26,4	16,4	7,76	43,1	18,2	24,1	14,5
Third	7,53	43,9	19,2	22,5	14,2	8,19	37,6	19,1	26,1	17	8,23	35,9	20,1	27,6	16,2
Fourth (Control)	7,39	45	20,7	20,7	12,1	7,95	39,2	19,7	26,3	14,5	7,49	38,6	17,7	27,6	15,9

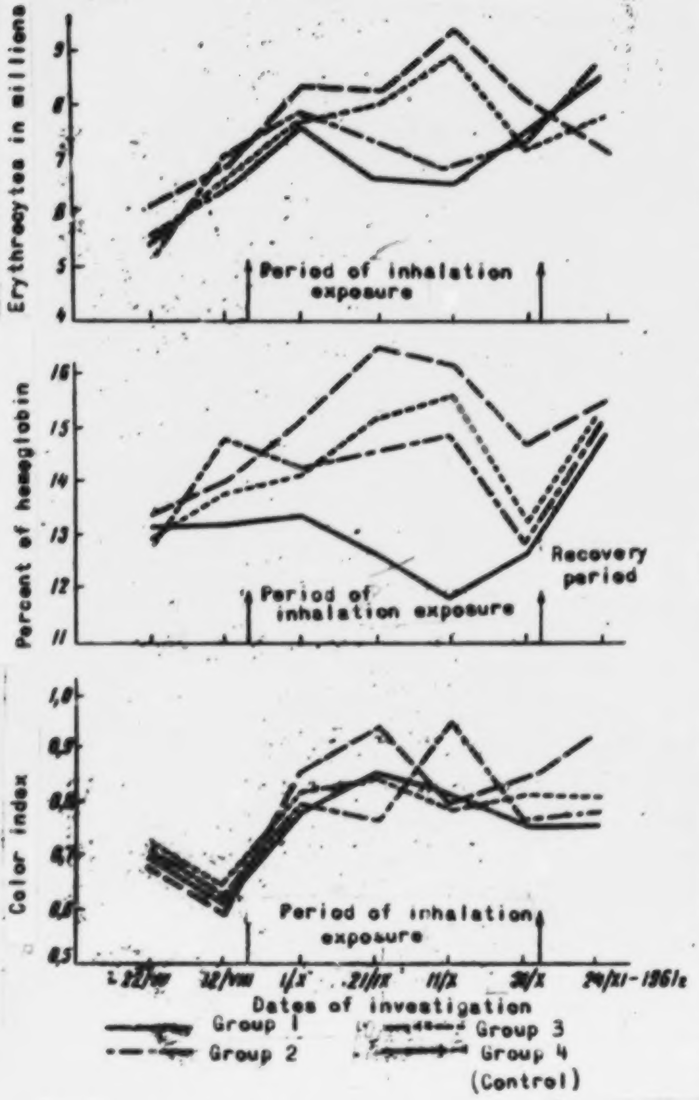

Fig. 6. Amount of erythrocytes, hemoglobin, color index, in rats during aniline vapor inhalation exposure

The third stage began with the 55th day of aniline vapor inhalation; during this period the number of erthrocytes in the blood of rats belonging to groups 1 and 2 rose probably as a compensatory reaction on the part of the organism to the effect of aniline vapor. Similar changes were found in blood hemoglobin concentrations. Prior to the aniline vapor inhalation blood hemoglobin in rats of all groups stayed within the limits 12.8 - 14.9 g %. A drop in the hemoglobin concentrations was noted in rats of group 1 begining with the 33rd day of the aniline vapor inhalation. It again dropped to 11.9 g % on the 53rd day of aniline vapor inhalation. Statistically significant changes were noted in rats of group 2, the absolute values of which were below those observed in rats belonging to group 1. The hemoglobin concentration of rats belonging to all groups receded to 14.6 - 15.5 g % on the 20th day of the recuperation period.

No statistically significant changes were noted in the color index in any rats under investigation. A significant anisocytosis, poikilocytosis, anisochromia, and an increase in the number of reticulocytes were noted in rats of group 1, and partly of group 2, during their inhalation of air containing aniline vapor. However, these changes did not persist, and on the 20th day of recuperation they completely disappeared. A study of the blood of rats chronically inhaling air containing 3 and 0.3 mg/m^3 of aniline vapor showed the presence of a normochromic anemia. In this respect results of the present author agreed with the similar results obtained by other investigators. No hyperglobulinemia was observed in rats which inhaled air containing aniline vapor.

Further investigations were compared with a study of atmospheric air conditions in the vicinity of a phenol producing plant in which aniline was used in the production of neosone D. (A USSR trade-name for which no USA equivilant was found.) Highest aniline concentrations were found in the exhaust air conduits. Many of the production processes in this plant were equipped with conduits through which aniline vapor was returned for reuse; nevertheless, much of the aniline vapor found its way into the atmospheric air probably due to the fact that some processes employed in this plant were conducted at high temperatures. Aniline vapor may have been discharged into the atmosphere through leakage points, during repair making and through other unorganized points of aniline vapor discharge. Samples were collected during the spring and summer seasons at different times of the day at distances ranging between 100 and 1000 meters from the plant on the lee side, in the direction of the prevailing work. A total of 188 samples were thus collected and analyzed by the M. V. Alekseeva and R. V. Khokhlova method eliminating the interfering results of ammonia as much as possible. Resulting data presented in Table 4, show that at 750 meters from the source of ventilation air discharge, three of the 35 air samples contained aniline vapor in excess of the MAC, that is in excess of 0.05 mg/m^3. At 1000 meters from the point of ventilation air discharge, none of the 44 air samples tested contained aniline vapor exceeding the MAC. On the basis of results obtained as indicated in the preceding paragraphs it is recommended that the sanitary protection zone for the plant producing neosone D under conditions prevailing in the present investigation should be not less than 1000 meters wide.

Table 4

MAXIMAL SINGLE ANILINE VAPOR CONCENTRATION IN ATMOSPHERIC AIR IN THE PROXIMITY OF THE PHENOLIC PLANT DURING THE SPRING AND SUMMER SEASONS OF 1962

Meters from source of discharge	No. of air samples tested		Aniline concn. in mg/m^3			No. of air samples with mg/m^3-indicated concentrations			
	Total	Below the method sensitivity	Maximal	Minimal	Average	From 0.50 to 0.31	From 0.30 to 0.11	From 0.10 to 0.05	Below 0.05
100	33	—	0,5059	0,0223	0,1216	1	13	12	7
300	40	—	0,1344	0,0048	0,0398	—	1	11	28
500	36	—	0,0956	0,0070	0,0394	—	—	14	22
750	35	3	0,0795	Traces	0,0215	—	—	3	32
1000	44	1	0,0324	Traces	0,0135	—	—	—	44

Conclusions

1. A study was made of the biological effect of low aniline vapor concentrations in the atmospheric air. Results indicated that aniline possessed protoplasmic poison properties which affected all the organs and systems of the experimental animals, predominantly the central nervous and blood systems.

2. The minimal aniline odor perceptible concentration (threshold of odor perception) for most odor sensitive persons was 0.37 mg/m^3, and the maximal odor nonperceptible concentration was 0.35 mg/m$_3$.

3. The threshold of aniline vapor reflex effect of short exposure was determined by the electro and encephalographic method. Results showed that for most sensitive persons the threshold effect was at 0.07 mg/m^3.

4. Exposure of white rats to the effect of aniline vapor under chronic conditions produced the following results: (a) 3 mg/m^3 of aniline vapor affected the muscle-antagonists chronaxy ratio, enhanced the blood cholinesterase activity, raised the rate of coproporphyrin elimination with the urine, lowered the level of total albumin, raised the concentration of α- and β- blood serum globulins, lowered the albumin-globulin, the albumin -α- globulin and the albumin -β- globumin coefficients, elicited the appearance in the blood of methemoglobin and Heinz bodies and the appearance of chromic anemia; (b) aniline in 0.3 mg/m^3 disturbed the muscle antagonist chronaxy ratio, enhanced blood cholinesterase activity, lowered the total albumin and raised the concentration of blood serum -globulins, caused the appearance of methemoglobin and Heinz bodies paralleled by a slight anemia. These changes appeared later than in rats which inhaled air containing 3 mg/m^3 of aniline vapor, and were not as clearly differentiated; normalization of the symptoms in such rats appeared at an earlier date; (c) 3 mg/m^3 and 0.3 mg/m^3 of aniline vapor caused functional disturbances produced pathomorphological changes in the internal organs and systems of the experimental rats. These changes were more pronounced in rats of group 1.

5. It is proposed that 0.03 mg/m^3 of aniline vapor in atmospheric air be adopted as the maximal single and 24 hour average concentration, since it elicited no changes in the organism under experiemntal conditions.

6. Atmospheric air pollution with aniline vapor was found at considerable distances from the original source of its discharge into the atmosphere.

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SOME METHYLMETHACRYLATE CHARACTERISTICS AS AN ATMOSPHERIC AIR POLLUTANT

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Progress in chemistry is intimately related to the production of new compounds which frequently cause serious air pollution. Methyl methacrylate is a methacrylic acid ester. It is a colorless fluid having a characteristic ester odor. Its b. p. is 100.3°, melting point 48°, vapor tension 35 mm of mercury column, and volatility of 192 mg/li. The substance is 1/10 as volatile as ether. It is soluble in most organic solvents, but is practically water insoluble. Methyl methacrylate is easily polymerized It is widely used in the production of organic glass. Methyl methacrylate is produced commercially with the aid of hydrocryanic acid or its salts. The intermediate product, acetone hydrocyanate, is treated with sulfuric acid at 100 - 110° in alcoholic medium which converts the mixture into methylmethacrylate. Polymerization of methyl methacrylate can be arrested by the addition of 0.005 - 0.01% of hydroquinone or pyrogallol. All plants that use the production of which requires the presence of methylmethacrylate at any production stage can be regarded as potential sources of atmospheric air pollution with this compound. L. F. Glepova studied atmospheric air pollution in the vicinity of a plant which synthesized methylmethacrylate and found the presence of this ester in the air at 2 km in 0.4 - 0.89 mg/m^3 concentrations.

The toxicology of methylmethacrylate had not been adequately investigated, and such studies as had been conducted were concerned with relatively high concentrations of the ester. Results indicated that the substance possessed narcotic and general toxic properties. Thus, exposure of guinea pigs and white rats to the inhalation of air containing 19 mg/li of the substance for 2 hours killed the experimental animals. The toxicity picture was generally characterized by an initial state of stimulation followed by depression of the nervous activity in which state the animals usually died. In the case of man 0.1 - 1.0 mg/li concentrations of the substance caused irritation of the upper respiratory tract mucosa. 0.13 - 0.25 mg/li was determined as the concentration of threshold odor perception. However, such data are now under serious doubt. Medical examination of workers employed in plants producing organic glass disclosed well defined functional disturbances in the form of high stimulation, easy fatigability lack of appetite, and continuous nasal secretion. Fifty percent of the examined workers showed symptoms of hypotonia and disturbed liver function. B. B. Karpov proposed 0.05 mg/li of the ester as the orientation concentration for the limit allowable concentration of this poison in the air of production premises.

Determination of low methylmethacrylate concentrations in the atmospheric air was made colorimetrically by the Gronsberg method, the sensitivity of which was raised considerably (up to 0.001 mg and 1 ml) in the present investigation. In the first stage of the investigation the effect of short time exposure to the substance was investigated, and the threshold non-odor perception concentration and eye sensitivity to light were determined by the method of electroencephalography. Fourteen non-smoking test persons, 18 to 59 years of age, of normal perceptive senses were used in the investigation. Duplicate tests were made with identical concentrations twice daily at 3 to 4 hour intervals on two to 5 successive days. Following this the methylmethacrylate concentrations were gradually lowered until negative perceptive results were reported in most occasions by most of the test individuals. Specific perceptive thresholds were recorded by individual investigators.

The following concentrations had been studied: 1.8, 1.2, 0.9, 0.5, 0.2, and 0.12 mg/m^3, making a total of 304 odor perception determinations. On the basis of obtained results it was computed that the minimal perceptible concentration of the tested substance ranged within 1.2 - 0.2 mg/m^3. The threshold odor perception concentration in most sensitive persons was 0.2 mg/m^3, as shown in Table 1. This value was one-thousandth of the value obtained for methylmethacrylate threshold odor perception by the statistical method of Ponomareva-Astrakhantseva and B. D. Karpov in 1954.

Table I

DETERMINATION OF ODOR PERCEPTION THRESHOLD OF METHYLMETHACRYLATE

No. of test persons	Methlmethacrylate concn. in mg/m^3	
	Minimal perceptible	Maximal non-perceptible
4	0,2	0,1
4	0,5	0,2
1	0,9	0,5
5	1,2	0,9

Curves of eye adaptation to the dark were determined in three persons 20 to 50 years of age, using adaptometer ADM. All persons were medically examined and found free from serious sensory defects opthalmologically and otherwise. The test individuals were subjected first to a 4 to 5 day training period after which the individual normal adaptation curves of eye sensitivity to the dark were established. The effect of methylmethacrylate vapor on persons' eye adaptation to the dark was then actually tested. Tests were made once daily for 40 minutes following a preliminary 3 minutes of readaptation. During the first 30 minutes eye sensitivity to light was tested every 5 minutes. Final observation was made on the 40th minute, and at the appearance of highest rise in eye sensitivity to the light, which usually occurred between the 15 and 20th minutes, the test individual was given to inhale a known methylmethacrylate concentration. Determinations with specific methylmethacrylate concentrations were conducted on three successive days. In this way concentrations of 0.25, 0.20, and 0.15 mg/m^3 of the methylmethacrylate were tested. Results of such tests conducted with test individual P, are presented as curves in Figure 1. Up to the fifteenth minute, i.e., up to the inhalation of fresh air, or of methylmethacrylate, by each of the three test individuals the results were practically identical, and only after inhalation of the tested methylmethacrylate concentration on the 20th minute did the indicators vary profoundly. Methylmethacrylate in 0.25 mg/m^3 concentration elicited a sharp drop in the curve of dark adaptation in all three test individuals on the 20th minute as compared with the control curves due, most probably, to the inhibition effect elicited in the brain cortex. In 0.15 mg/m^3 concentration methylmethacrylate had no effect on the curve of eye sensitivity to light in any of the three test individuals, similar to the case of fresh air inhalation. This is shown by data presented in Table 2. Thus, the methylmethacrylate vapor threshold effect on eye sensitivity to light was determined at 0.3mg/m^3, which is at the level of odor perception threshold of most sensitive individuals so far as odor perception was concerned. The odor perception threshold in test individual P was determined at 0.5 mg/m^3 concentration. Accordingly, in the case under consideration there occurred a change in eye sensitivity to light of the optical analyzer at concentrations not detected by the odor percepting mechanism.

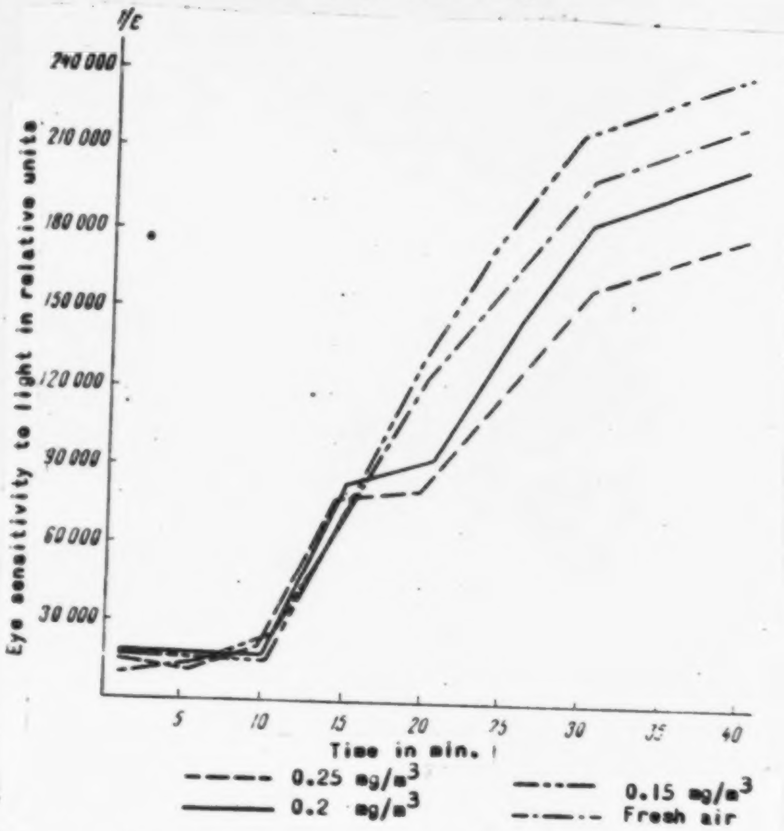

Fig. 1. Effect of different methylmethacrylate concns. on eye sensitivity to light of female R.

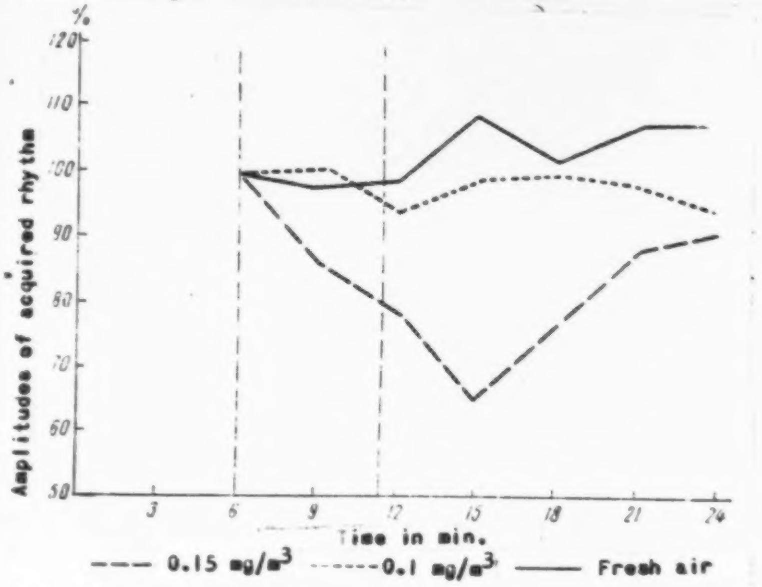

Fig. 2. Changes in the amplitude of electroencephalogram curves of rhythms acquired during methylmethacrylate vapor inhalation by female Ea.

A. D. Semenenko and others had shown that the method of functional electroencephalography was sensitive enough to detect changes in quantitative indexes reflecting the state of the central nervous system under the effect of toxic substances in subthreshold odor perception concentrations. The present author also used such a methodical procedure. Observations were conducted over 5 practically normal individuals kept in a standard chamber. The general procedure was the same as described on previous occasions elsewhere. Records of the electrical brain activity were made with an 8-lead Kaizer electroencephalograph. Records were made simultaneously of respiration and electrocardiographically of heart activity. Duplicate records were made of the individuals' characteristic background, after which tests were made with 0.15 or 0.1 mg/m^3 concentrations of methylmethacrylate. The poisonous vapor was inhaled for 5 minutes. On the 3rd minute of this period records were made of the adaptation rhythm. A 4th curve was recorded 10 minutes after the poisonous vapor inhalation was discontinued. Rhythm curve records were taken every two minutes until the original rhythm level reappeared. Tests were conducted in triplicate. Results shown by curves in Figure 2 indicated that methylmethacrylate in 0.1 mg/m^3 concentration had no effect on the character of the normal encephalogram. The 0.1 mg/m^3 methylmethacrylate concentration appeared to act as a subthreshold concentration by the three methods of investigation in relation to man. It is proposed that this concentration should be adopted as the maximal single methylmethacrylate concentration in the atmospheric air. Determination of average 24 hour maximal methylmethacrylate in atmospheric air was determined by the chronaxymetric method with mice exposed to the inhalation of different methylmethacrylate concentrations for 60 days.

Tests were made with four groups of rats consisting of 16 specimens each. Rats of group 1 were exposed to the inhalation of air conctaining 10 mg/m^3 of the methylmethacrylate, which is 1/5 the concentration of the MAC adopted for air of working premises; rats of group 3, were exposed to the inhalation of air containing 0.1 mg/m^3 of the methylmethacrylate, equivalent to the concentration above proposed for the MAC. Rats of group 2 were exposed to the inhalation of air containing 1 mg/m^3 of methylmethacrylate; rats of group 4 served as controls. In the course of preliminary 30 days tests were made to determine the background indexes of the experimental rats. Thus, determinations were made of muscle antagonists chronaxy ratios, rate of coproporphyrin elimination with the urine, changes in blood cholinesterase activity, etc. Records were also kept of changes in the course of body weight. Reasonable constancy was maintained in the methylmethacrylate concentrations in the experimental chambers. Throughout the period of experimental inhalation the rats remained active and showed no unfavorable effects resulting from the vapor inhalation. However, at the end of the experimental periods the record showed that rats of group 1 suffered a statistically significant loss in body weight which persisted through the recuperation period. Rats of groups 2 3 showed no statistically significant loss in body weight, as was the case with rats of the control group. This is clearly shown in curves illustrated in Figure 3.

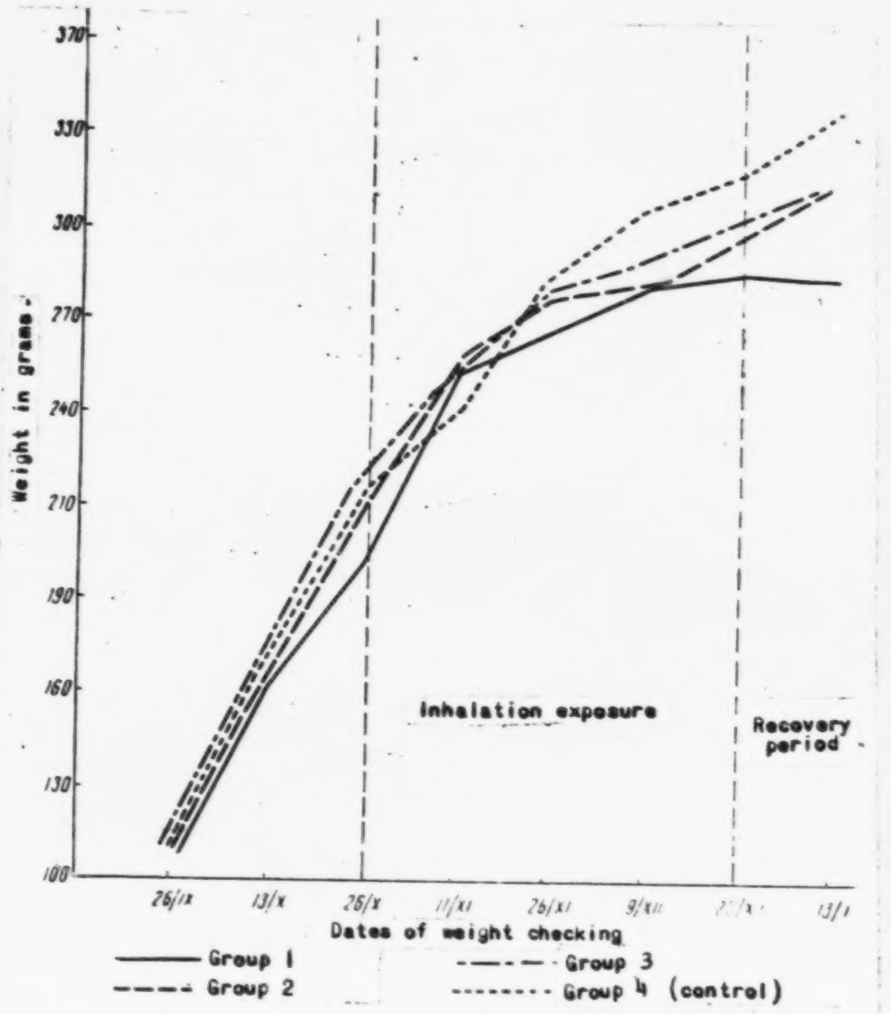

Fig. 3. Changes in average weight in rats of different groups.

Changes in muscle antagonists chronaxy and in the rheobase were determined weekly using electric chronaximeter ISE-01 in 5 rats of each group. At the same time, and under identical conditions, beginning with the fourth week of the inhalation exposure there appeared statistically significant changes in the muscle antagonists, motor chronaxy of rats belonging to group 2, as shown in curves illustrated in Figures 4 and 5. The motor chronaxy ratio of rats belonging to group 3 throughout the chronic experiment retained its original value, as shown by curves in Figure 6.

Changes in the rate of corproporphyrine elimination with the urine were determined next. Rate of diuresis in the 5 test rats varied between 58 and 133 ml, depending upon the animals age. Rate of corproporphyrin elimination with the urine of rats belonging to the control group gradually rose during the experiment, amounting to 3.7 μ at the end of the test. This was undoubtedly due to the growth of the control rats. Rate of coproporphyrin elimination in rats of group 3 ran a course parallel to that noted in the control rats. Rate of coproporphyrin elimination with the urine by rats belonging to group 1, which inhaled air containing 10 mg/m^3 of the methylmethacrylate vapor, showed a reduction beginning with the second week of the polluted air inhalation as compared with the control data.

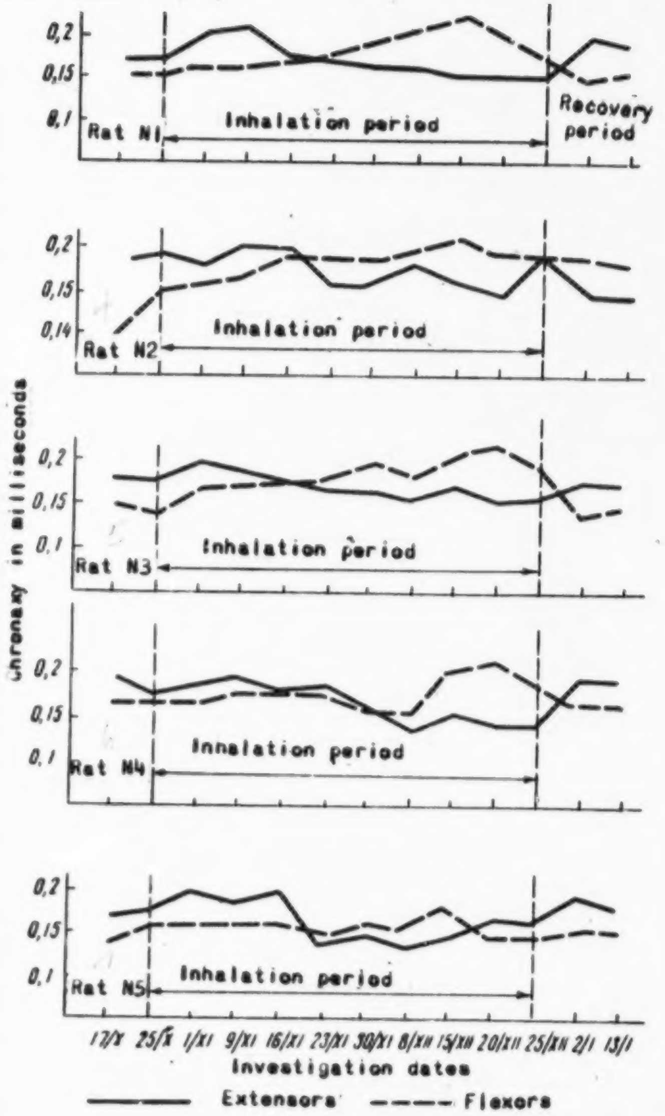

Fig. 4. Flexer and extenser chronaxy indicators in rats of Group one

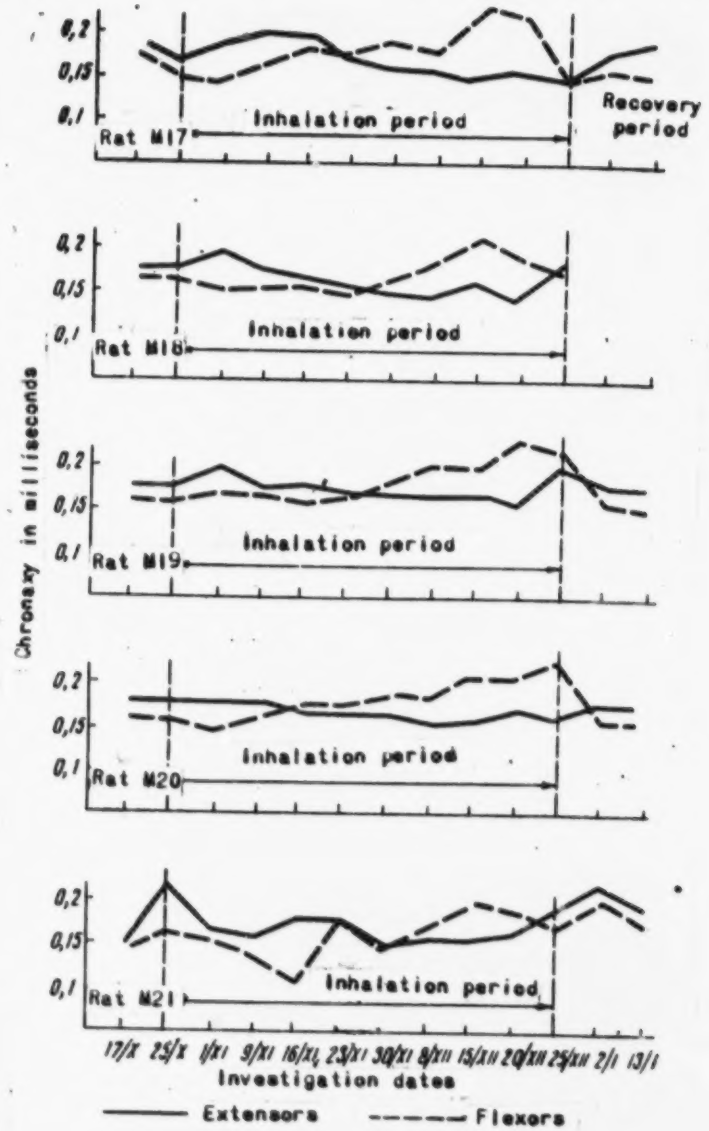

Fig. 5. Flexor and extensor chronaxy indicators in rats of Group 2.

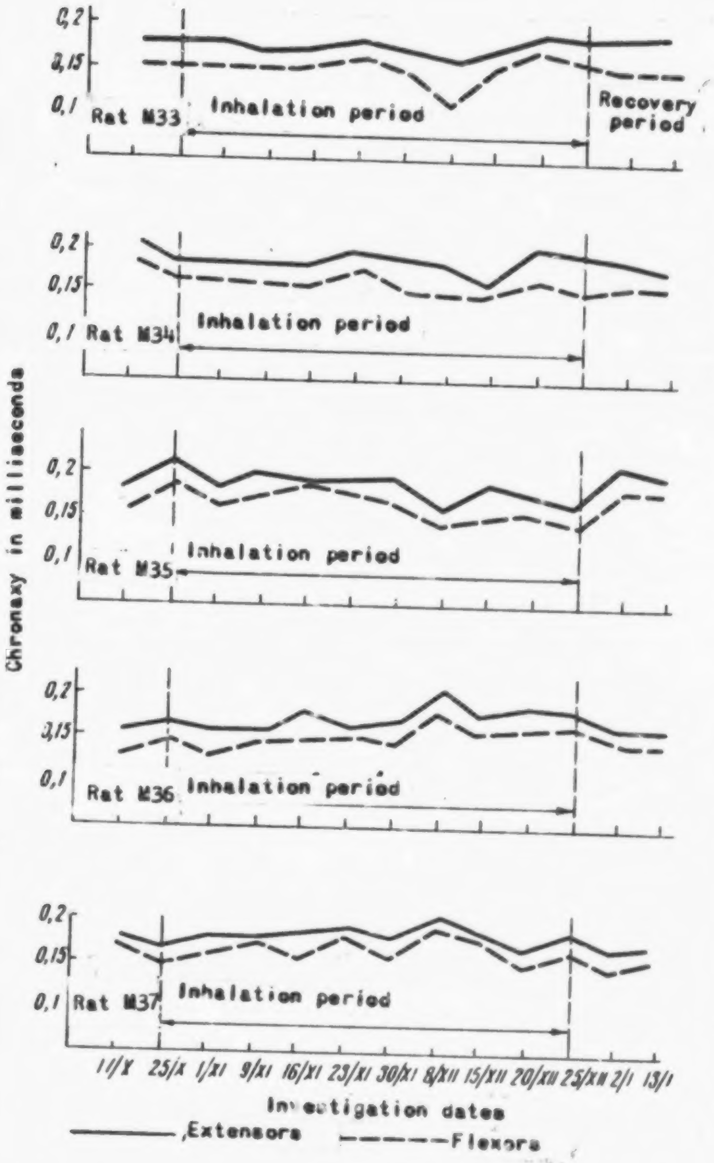

Fig. 6. Flexor and extensor chronaxy indicators in rats of Group 3.

The lowest coproporphyrin elimination was noted on the last date of the experiment in one rat belonging to group 1, which amounted to 0.79 μ or 0.28 μ per hundred g of rat's body weight. Some reduction in the rate of coproporphyrin elimination rate with the urine was noted in rats of group 2, which inhaled air containing 1 mg/m^3 of the methylmethacrylate. It should be noted, however, that reduction in the coproporphyrin elimination with the urine appeared as a temporary phenomenon and ultimately returned to its original level. Comparing data obtained on the rate of coproporphyrin elimination through the experimental period, it can be seen that one rat of group 1 eliminated throughout the experimental period 5.3 μ of the coproporphyrin as compared with 15 μ eliminated by an individual rat of the control group. The value was verified statistically as reliable.

Changes in whole blood cholinesterase activity were determined by the Pokrovskii - Martynova method. Cholinesterase activity was determined in 5 rats of each group fortnightly. Prior to the inhalation of air containing methylmethacrylate the original hydrolysis time of the enzyme ranged between 37 and 42 minutes. It extended to 47 minutes in rats of group 2 beginning with the 2nd week of inhaling air containing methylmethacrylate. At the end of the experiment the time was extended to 66 minutes, as shown by curves presented in Figure 8. The same was noted in the case of rats belonging to group 3. Here, however, the time extension was not as pronounced as in the case of rats belonging to group No. 1. No statistically significant or verifiable changes in the enzyme reaction time were noted in rats belonging to group 3. Pathomorphological investigations showed that chronic inhalation of air containing methylmethacrylate in 10

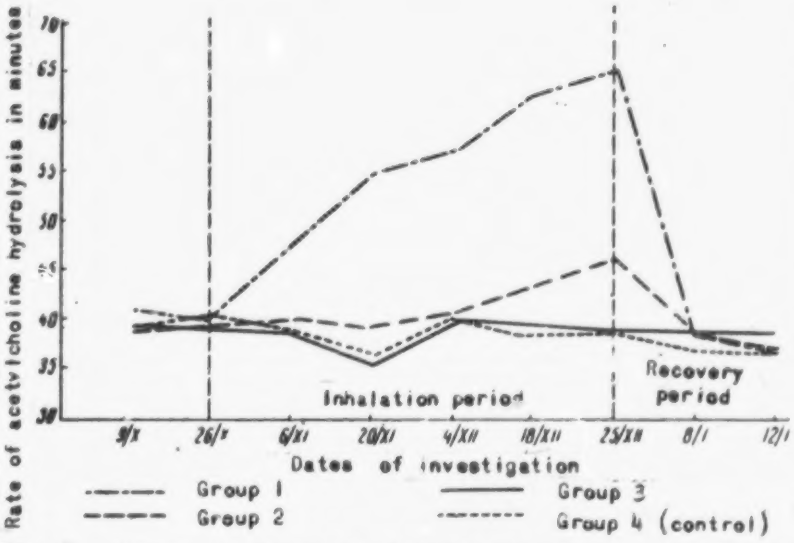

Fig. 8. Changes in whole blood cholinesterase activity in rats of different groups

mg/m^3 concentrations directly affected lung tissue of white rats, as shown by the appearance of punctate pneumonia, catarrh of the finer bronchi, dystrophy changes in kidney tissue, splenic dystrophy and other pathologic changes.

At the end of two weeks recuperation the vascular effects disappeared, and the dystrophic changes in the central nervous system considerably improved. Pathomorphologic changes observed in rats belonging to group 2 appeared in isolated cases and were rather insignificant. No pathomorphological attacks were noted in the internal organs or in the central nervous system of rats belonging to experimental group 3. Thus, continued exposure of white rats to methylmethacrylate vapor inhalation for 60 days produced notable results only in rats belonging to group 1, which inhaled air containing 10 mg/m^3 of the methylmethacrylate. No unfavorable pathomorphological effects were noted in the internal organs and central nervous system of rats belonging to group 3. Accordingly, it is recommended that the 24 hour average allowable methylmethacrylate concentration in atmospheric air should be set at 0.1 mg/m^3.

A hygienic survey was then conducted for the determination of the degree and extent of atmospheric air pollution with methylmethacrylate which prevailed in the proximity of Orekhovo-Zuevskii plant "Karbolit" for the production of synthetic organic glass. Air samples were collected during 2 - 29 November of 1962 at 100 to 1000 km from the plant on the lee side from the source of methylmethacrylate discharge into the atmospheric air. The air was aspirated through two successive absorbers each containing 5 ml of 2.5% $NaOH$, at the rate of 30 li/hr. Volume of the aspirated air varied between 10 and 100 li. Air samples were collected at different hours of the day. The air temperature during the aspiration usually varied between + 10 and - 4°; barometric pressure varied between 743 and 765 mm of mercury; average rate of wind velocity was 3 m per sec. During the first part of air sample collection the weather was dry and clear, later the skies were slightly cloudy, the air was frosty accompanied by some precipitation. Results of quantitative methylmethacrylate determinations are presented in Table 3. Data in the Table show that the atmospheric air in the proximity of the organic glass producing plant was systematically polluted with methylmethacrylate vapor as a result of leakage from faulty conduits of the

Table 3

DIFFERENT METHYLMETHACRYLATE CONCENTRATIONS IN THE AIR SURROUNDING THE ORGANIC GLASS PLANT

Meters from source of discharge	No. of samples	Maximal concn. in mg/m^3	Concn. distribution in samples mg/m^3					
			Exceeding 2	2—1	1.05	0.5—0.1	0.1	Below 0.1
100	28	4,1	12	11	4	1	—	—
200	32	2,3	2	6	18	6	—	—
500	29	1,1	—	2	4	23	—	—
800	28	0,4	—	—	—	17	7	4
1 000	26	0,1	—	—	—	—	1	25

ventilation system, the technology of the plant's calcination processes were antiquated, the plant had no mechanization and no gas or vapor purification equipment. Only in all air samples collected at 1000 m from the plant was the concentration of methylmethacrylate below the maximal allowable concentration of 0.1 mg/m^3. The method used for the quantitative determination of methylmethacrylate lacked specificity since the presence of formaldehyde in the air interferred with the determination, and formaldehyde found its way into the surrounding air from neighboring plants producing phenol-formaldehyde resins. For this reason air samples were collected also for the quantitative determination of formaldehyde in the surrounding atmosphere. Thirty air samples were collected at distances varying from 100 to 1000 m from the investigated plant producing organic glass. The test procedure used was based on the chromotropic reaction. No formaldehyde was found in any of tested air samples.

Conclusions

1. The methylmethacrylate odor perception threshold was established at 0.2 mg/m^3; threshold of reflex effect in relation to eye sensitivity to light was established at 0.25 mg/m^3; threshold effect on electrical brain activity was established at 0.15 mg/m^3; and inactive concentration was determined at 0.1 mg/m^3.
2. Continuous chronic inhalation of air containing 10 and 1.0 mg/m^3 of methylmethacrylate vapor elicited in experimental rats shift in the normal muscle antagonists chronaxy ratios, caused a drop in the blood cholinesterase activity, lowered the rate of coproporphyrin elimination via the urine. In 0.1 mg/m^3 concentration methylmethacrylate elicited no shifts in any of the above enumerated indexes under similar experimental conditions.
3. On the basis of the above findings it is recommended that the MAC for methylmethacrylate in atmospheric air be set at 0.1 mg/m^3.
4. Atmospheric air in the proximity of an organic glass producing plant consistently contained methylmethacrylate as a pollutant. This unhygienic condition can be eliminated by preventing unorganized methylmethacrylate leakage, by installing gas purifying equipment at strategic production points, by moderizing the production technology, and by other similar hygienic improvements.

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1. Порог обонятельного ощущения метилметакрилата равен 0,2 мг/м^3, порог рефлекторного изменения световой чувствительности глаза — 0,25 мг/м^3, порог действия на электрическую активность головного мозга — 0,15 мг/м^3. Недействующей концентрацией является 0,1 мг/м^3.

2. Концентрация метилметакрилата 10 и 1 мг/м^3 в процессе хронической непрерывной затравки ингаляционным методом вызывает у подопытных белых крыс изменения правильного соотношения хронаксии мышц-антагонистов, снижение активности холинэстеразы и уменьшение выделения копропорфирина с мочой. Метилметакрилат в концентрации 0,1 мг/м^3 при тех же условиях не вызывает аналогичных изменений в организме животных.

3. На основании проведенных исследований рекомендуется установить предельно допустимые концентрации метилметакрилата в атмосферном воздухе (максимальную разовую и среднесуточную) на уровне 0,1 мг/м^3

4. Атмосферный воздух вокруг производства органического стекла систематически загрязняется метилметакрилатом. Основное внимание в борьбе с этим загрязнением должно быть обращено на рационализацию технологического процесса, усовершенствование и герметизацию аппаратов и коммуникаций, разработку способов очистки газов, выбрасываемых вентиляцией.

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MAXIMAL ALLOWABLE HEXAVALENT CHROMIUM CONCENTRATION IN ATMOSPHERIC AIR

From the Sverdlovsk Institute of Labor Hygiene and Occupational Diseases and from the Department of Community Hygiene, the Central Institute of Post-Graduate Medicine

E. F. Kuperman

Production of chromium compounds is an important branch of USSR national production. There are over one-hundred production industries which in one way or another use chromium compounds of the dii-tri-, tetra-, penta and hexavalent type. Most toxic of all the chromium compounds are the hexavalent types, as for instance, chromic anhydride, monochromates and bichromates, which possess local and general toxic properties. Attention has been centered of late on the relatively frequent occurrence of lung cancer among workers engaged in the different branches of chromic industry. Modern processes for the industrial production of chromates and their derivatives are usually resultant in the formation of hexavalent chromium aerosols which are discharged into the atmospheric air. Air may also become polluted with chromium in the production of ferric alloys and by electrical chrome plating. Little information was found in the literature on the subject of air pollution with chromium compounds. The present author found only one reference published by Bourne in 1950. No information was found in the literature dealing with the effect of chromium on the health of residents living in the proximity of different branches of the chromium industry. Accordingly, a new and detailed study of this subject had to be initiated to find a basis for the adoption of a rational MAC for chromium in atmospheric air. In this connection, hexavalent chromium in atmospheric air was determined quantitatively by the 1954 method of S. Gurvits and G. I. Sergeeva, the sensitivity of which had to be increased.

Hexavalent chromium was determined by a photocolorimetric method based on the well known diphenyl carbazide reaction. Sensitivity of this method was 0.0001 mg per 1 ml. The studied atmospheric air was aspirated consecutively through number 2 membrane filter or through FPP-15 installled into a Rukhter absorber which contained 10 ml of distilled water. Usually 30 li of the air was aspirated through this assembly at the rate of 1 li/min. Aerosol of the hexavalent chromium condensate was obtained by heating chromium and hydride in an electrical tubular heater up to the point of melting that is, up to 196 - 250°. An aliquot of this, usually a small quartz combustion boat containing lg of chromium was placed into tubular furnace, as shown in Figure 1. Temperature stability was maintained by a thermostat connected to a recording electrical potentiometer. The desired chromium aerosol concentration was maintained through contolled temperature inside the electrical furnace and other pertinent factors.

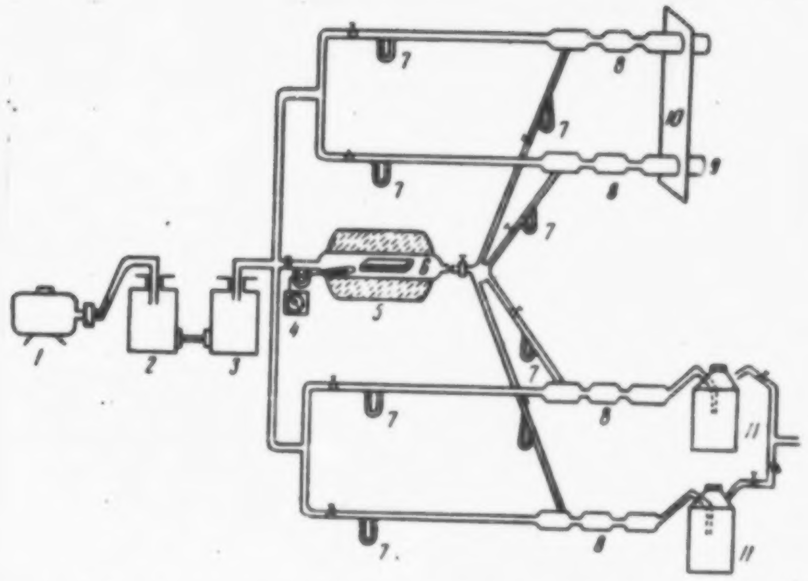

Fig. 1. Plan of the experimental installation.
1 - Air blower; 2 - Silicagel; 3 - Activated charcoal; 4 - Electropotentiometer; 5 - Electric furnace; 6 - A quartz dish; 7 - Flow meter; 8 - Mixer; 9 - Glass cylinder; 10 - Shield; 11 - Inhalation exposure chamber

In this way the air containing the hexavalent chromium aerosol condensate was directed into the mixers through which air passed at the rate of 20 li/min. The gas mixture with a known chromium aerosol concentration was run into glass cylinders and from there into the experimental inhalation chamber.

Studies began with the determination of concentrations representing threshold irritation of hexavalent chromium. Tests were conducted by the standard procedure previously described elsewhere, using 10 ostensibly normal test persons. In the present study 260 such determinations were made representing 12 different chromium aerosol concentrations ranging in descending order from 40 to 1.5 μ/m^3. Results established that short periods of inhaling air containing 10 - 24 μ/m^3 of chromium sharply irritated the nares. Lower concentrations produced slight irritation of the upper respiratory tract. Minimal perceptible hexavalent chromium concentrations in the test persons ranged within 4 and 2.5 μ/m^3. The threshold concentration for most sensitive persons averaged 2.5 μ/m^3, as indicated by data presented in Table 1.

Determination of chromium threshold reflex effect on eye sensitivity to light was conducted by the method of dark adaptation, using ADM adaptometer and three test persons whose concentration of chromium irritation threshold was previously determined. Dark adaptation was conducted by first using fresh air followed by mixtures of air with chromium aerosol in 2.5 and 1.5 μ/m^3 concentrations. Fresh air and different air and chromium aerosol mixture was delivered to the nostrils in the 15th minute of dark adaptation for 4.5 minutes. Each concentration was tested on 3

Table 1

DETERMINATION OF THRESHOLD CHROMIUM AEROSOL IRRITATION EFFECT.

No. of observations	Concn. of hexavalent chromium in mg/m^3	
	Minimal perceptible	Maximal non-perceptible
2	4,0	3,0
5	3,0	2,5
3	2,5	1,5

successive days. Sixty such determinations were performed. Curves in Figure 2, and data in Table 2, show that a brief period of hexavalent chromium aerosol inhalation in 2.5 μ/m^3 concentration elicited in all test persons an enhanced effect on the 20th minute followed by a lowered sensitivity of the eye to stimulation by light. On the 40th minute dark adaptation failed to return to its normal level. Statistical evaluation indicated that results obtained were significant. Thus, it was established that 1.5 μ/m^3 of chromium aerosol concentrate had no effect on the functional response of the eye. It was also established that the threshold reflex effect on the functional state of the brain cortex in the case of chromium aerosol as determined by the adaptometric method coincided with the concentration of its irritation effect. Evaluating the results obtained in the study of acute hexavalent chromium effect on the organism of man, it can be stated that 1.5 μ/m^3 of the poison in atmospheric air could be regarded as of sub-threshold value. This concentration is proposed by the present author as the maximal single allowable hexavalent chromium concentration in atmospheric air.

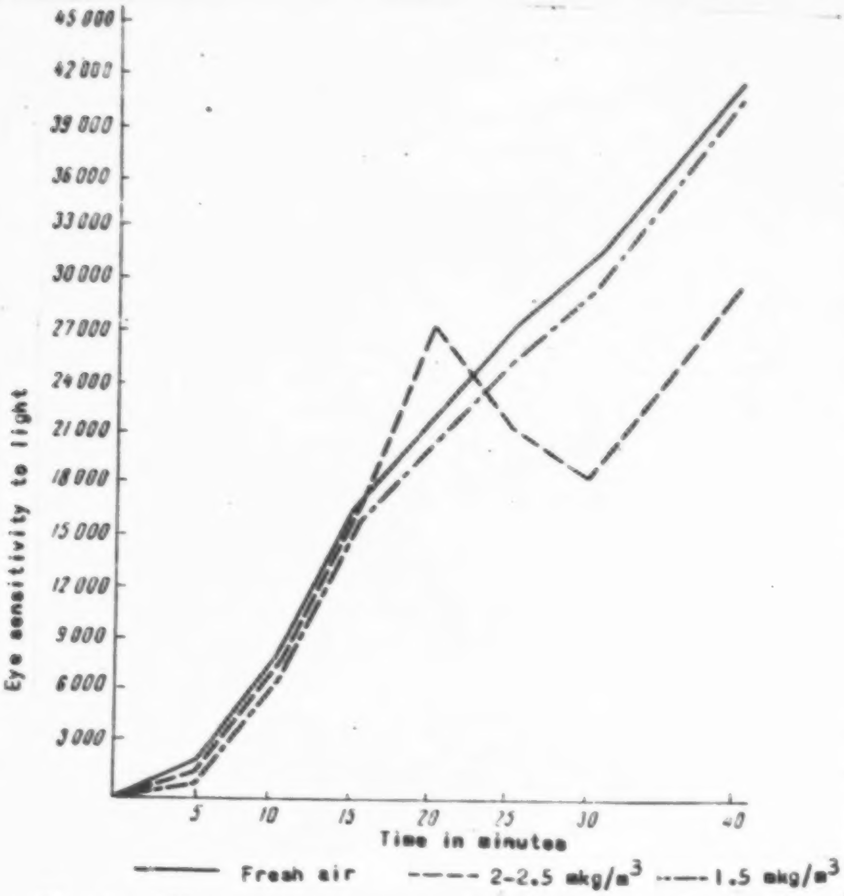

Fig. 2. Effect of different chromium concns. on eye sensitivity to light of Female L.

Table 2

AVERAGE VALUE OF EYE SENSITIVITY TO LIGHT DURING INHALATION OF DIFFERENT CHROMIUM CONCENTRATIONS IN PERCENT OF 15TH MINUTE VALUES

Initials of test person	20th min.			25th min.		
	Fresh air	Chromium in mkg/m^3		Fresh air	Chromium in mkg/m^3	
		2.5	1.5		2.5	1.5
A	150	198(a)	149(в)	205	184(a)	205(в)
G	118	266(a)	118(в)	205	237(a)	129(в)
L	129	162(a)	124(в)	160	128(a)	153(в)

Note: a - reliable result with probability of 99.9% or higher
b - result not reliable

Other stages of this investigation were concerned with the effect of low hexavalent chromium concentrations under chronic experimental conditions. In such experiments rats were exposed to the inhalation of air containing hexavalent chromium 20 hours daily for 90 days. Experiments were conducted with 45 white male rats weighing 90 to 120 grams. The rats were divided into 3 groups of 15 specimens each. Rats of group 1 were exposed to the effect of air containing 30 μ/m^3 of the chromium aerosol, which was equivalent to 1/3 of the concentration established for air of working premises; rats of group 2 were exposed to the inhalation of air containing 1.5 μ/m^3, or the equivalent of the proposed maximal single concentration; rats of group 3 served as controls. The system for producing hexavalent chromium aerosol condensate and its delivery to the experimental exposure chamber and means by which constancy of the mixture was controlled, was the same as described elsewhere. Air samples for purposes of analysis were collected four times daily. The average chromium concentration in chamber No. 1 was 29 ± 0.3 μ/m^3; it was 1.51 ± 0.026 μ/m^3 in the second exposure chamber. The hexavalent chromium aerosol particles in the gas mixture inhaled by the experimental animals in the exposure chamber consisted predominantly (90%) of particles up to 1 μ in diameter.

Through the entire course of rats' exposure to the inhalation of air containing hexavalent chromium aerosol concentrates rats behaved normally, and rats which inhaled air containing 30 μ/m^3 of the chromium aerosol showed a slight tendency to somnolence. The muscle antagonists chronaxy ratio and the rheobase were determined in 6 rats of each group. Results indicated that in rats which inhaled 30 μ/m^3 of the chromium the chronaxy ratio was enhanced, as shown in Figure 3. The value were statistically significant. At the end of the second recuperation week motor chronaxy returned to its original level. In some individual rats the results in connection with the chronaxy investigation were even more pronounced. Thus, curves in Figure 4 show that on the 9th week of the experiment there appeared a reverse chronaxy ratio in rats Nos. 4 and 5. In rats belonging to group 2, which inhaled air containing 1.5 μ/m^3 of the chromium, no changes in the normal muscle antagonists chronaxy ratios were noted. The composition and properties of peripheral blood

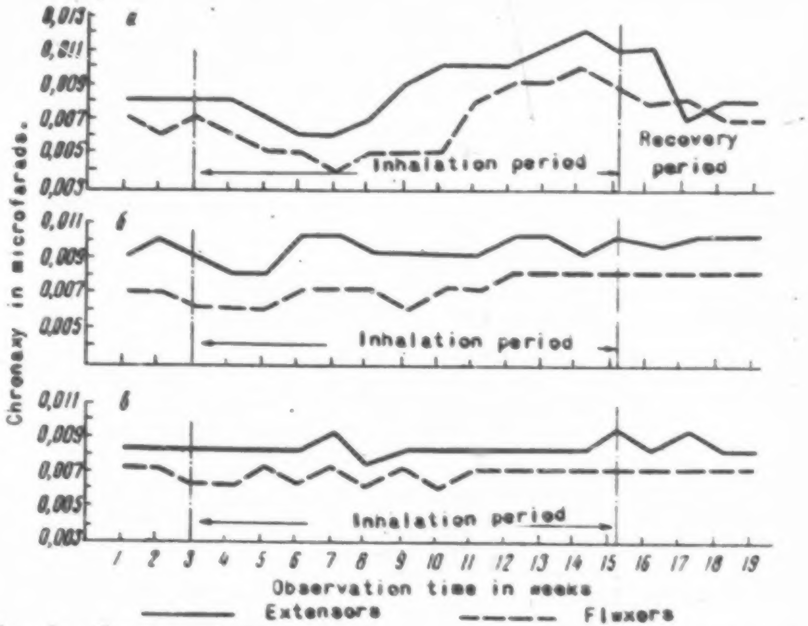

Fig. 3. Muscle antagonists motor chronaxy in rats of different groups

a - Group 1; b - Group 2; c - Group 3 (Control) manifested certain changes. Thus, the activity of blood carbonic anhydrase fell to a lower level in workers exposed to the effect of chromium.

The present author used the colorimetric method for the determination of carbonic anhydrase in whole blood which was developed by N. E. Ponomarenko. Activity of carbonic anhydrase was followed in three rats of each group. Prior to rats' exposure to the inhalation of air containing hexavalent chromium, anhydrase activity time averaged 8 - 9 seconds. In the middle of the experiment activity of this enzyme dropped to 15 - 20 seconds, as shown in curves of Figure 5. Changes in the carbonic anhydrase activity were of a temporary character. Statistical processing showed that the determined values were significant and reliable. In rats of group 2, exposed to the inhalation of air containing 1.5 μ/m^3 of the chromium, carbonic anhydrase activity was of the same intensity as in rats of the control group.

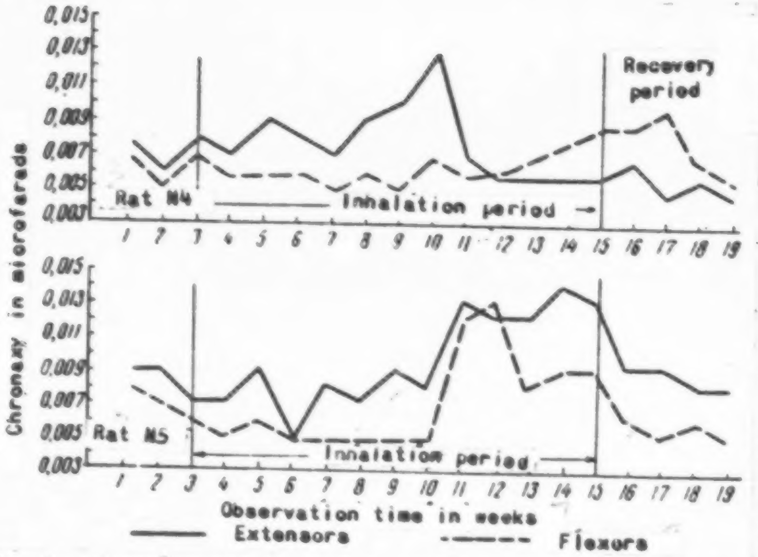

Fig. 4. Chromium effect on motor chronaxy of rats 4 and 5 of Group one

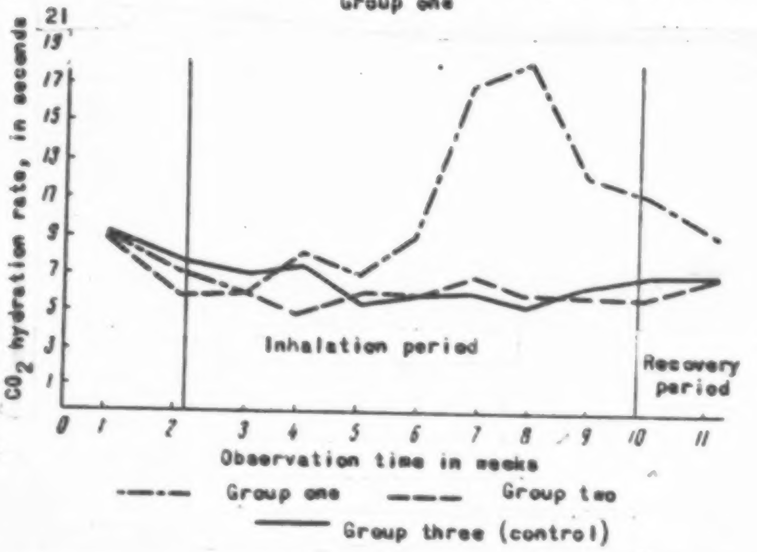

Fig. 5. Chromium effect on blood cholinesterase activity of different rat groups

No substantial changes were noted in the blood composition, the numbers of erythrocytes on leucocytes; hemoglobin concentration differed in no way from those found in rats of the control group. At the end of 30 days exposure to the inhalation of air containing 30 μ/m^3 of chromium, rats of group 1 showed an increase in the number of stabnuclear neutrophiles up to 6 - 8%, paralleled by the appearance of immature forms, neutrophilic myelocytes appeared in small numbers. The described changes in the blood picture reflected a shift to the left in the leucocytic formula pointing to disturbed functioning of the hemopoietic organs as a result of hexavalent chromium action. No morphologic changes were noted in the peripheral blood of rats exposed to the inhalation of air containing 1.5 mg/m^3 of the chromium.

Examination of tissues histomorphologically was conducted at the end of the third month of exposure to the inhalation of air polluted with different chromium concentrations. Changes were observed especially in tissues of the respiratory organs only in rats which inhaled air containing 30 μ/m_3 of chromium. The mucous membrane of the trachea was infiltrated with cellular elements of the lymphoid type, paralleled by an intensive hyperemia; there was evidence of other pathomorphologic changes. Lungs of rats belonging to this group showed signs of plethora, acute edema, induration of the interalveolar septi, and formation of air-free sections. Emphysema developed in rats of this group. There were many other pathomorpholgical changes. Rats of the group exposed to inhalation of air containing 1.5 μ/m^3 of the chromium showed no signs of pathologic tissue developments.

Thus, on the basis of chronic experimental data, it is proposed that the maximal 24 hour average hexavalent chromium concentration in atmospheric air should be set at 1.5 μ/m^3, which is also the maximal single concentration for this substance in atmospheric air. The latter elicited no functional changes in man or in laboratory animals. Results, thus, indicated that hexavalent chromium in 30 μ/m^3 of atmospheric air produced pathologic results in the experimental animals, indicating that the existing MAC of 100 μ/m^3 in atmospheric air must be reduced to a lower level.

The nature of atmospheric air pollution with hexavalent chromium compounds was studied in the proximity of 2 USSR most extensive plants producing chromium compounds. The chemical treatment of chromium ore rests basically on the principle of converting the trivalent raw material into hexavalent sodium and potassium nitrate. In the course of chromium ore treatment the air in the production premises becomes polluted with chromium vapor and is discharged into the atmospheric air by the exhaust ventilation system and by unorganized routes in the form of hexavalent substances, such as monochromates, bichromates, chromyl chloride, as aerosols or as vapors. In one of the plants the polluted air was passed through scrubbers which removed about 70% of the chromium pollutants before discharging it into the atmosphere. It has been established that about 700 kg of the hexavalent pollutants fell out from the discharged air at the height of 10 - 15 m in the course of a working day. Another plant was equipped with installations for the conversion of the crude chromium products into oxides of chromium with the aid of sulfuric acid solution in the presence of hexavalent chromium, which substantially reduced the rate of chromium pollutants' discharge into the atmospheric air. However, this process had the disadvantage of generating hydrogen sulfide, which was also discharged into the atmospheric air.

Air samples for the determination of hexavalent chromium were collected on the lee side of the plant within a 2 m radius from the point of the chromium discharge; 250 air samples were collected in the proximity of plant No. 1 and 100 air samples within the proximity of plant No. 2. Plots in Figure 6 show that highest air pollution with hexavalent chromium compounds was found within a radius 0.5 km from the plant, and amounted to 15 - 450 μ/m³, which is considerably in excess of the proposed MAC for this pollutant in atmospheric air. As the distance from the point of discharge increased above 0.5km, the pollution density rapidly abated. However, at 1.5 km from the point of discharge the chromium density in the atmospheric air was still in excess of its MAC. This was due partly to the specific characteristics of the local topography. At 2 km from the point of discharge pollution of the atmospheric air with hexavalent chromium dropped below the established MAC. Conditions of air pollution with hexavalent chromium were practically identical with the conditions prevailing in the vicinity of production plant 1.

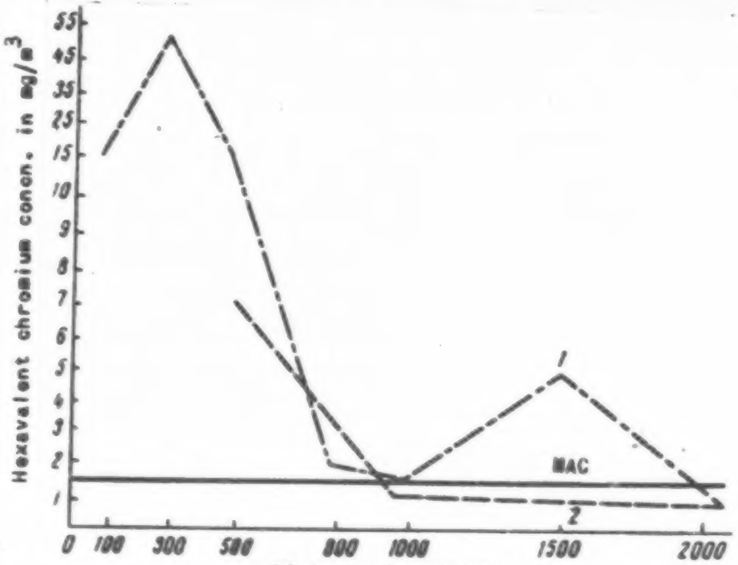

Fig. 6. Maximal single hexavalent chromium concns. in atmospheric air in the proximity of two plants producing chromium compounds

1 - Plant one 2 - Plant two

Conclusion

1. Threshold of hexavalent chromium irritation was established at 2.5 μ/m³. Threshold of chromium aerosol effect on the reflex activity and the functional state of the brain cortex was determined by the well known adaptometric method; for persons of high sensitivity such threshold was established at 2.5 μ/m³.

2. Exposure of rats to inhalation of air containing 30 μ/m³ of hexavalent chromium aerosol 20 hours daily for 90 days (chronic experimental conditions) affected the animals' muscle antagonists motor chronaxy, elicited a shift in the activity of carbonic blood anhydrase, and changed the morphological picture of the peripheral blood. Some pathomorphological changes in the internal organs and tissues were also noted.

3. No shifts from the normal indexes were noted under similar experimental conditions in rats exposed to the inhalation of air containing 1.5 μ/m³ of hexavalent chromium.

4. It is recommended that maximal single and 24 hour average concentrations of hexavalent chromium be set at 1.5 μ/m^3 computed on the basis of Cr_2O_3.

5. Atmospheric air in the proximity of the two investigated chromium plants was regularly polluted with hexavalent chromium, the concentration of which at 1.5 km from the point of the chromium discharge exceeded the officially adopted MAC.

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NEW EXPERIMENTAL DATA FOR THE DETERMINATION OF AVERAGE TWENTY-FOUR HOUR MAXIMAL CHLOROPRENE CONCENTRATION IN ATMOSPHERIC AIR

From the N. B. Akopian Institute of Epidemiology and Hygiene
of the Armenian Ministry of Health

A. V. Mnatsakanyan

The Committee for the Sanitary Protection of Atmospheric Air Affiliated with the Main USSR Government Inspection requested the present author to make a study of the effect of low chloroprene concentrations in the atmospheric air on different functions of the living organisms as a basis for the determination of maximal allowable chloroprene concentrations in the atmospheric air. Chloroprene is a nonsaturated hydrocarbon which is discharged into the atmospheric air by industrial plants producing or processing synthetic acetylene rubber. The study of such substances has recently become of particular importance with the progressing increase in the production of synthetic products using unsaturated organic compounds as the basis. Chloroprene, which is 2-chlorobutadiene-1, 3, is a colorless transparent fluid having a sharp specific odor; the substance possesses general toxic and local irritating properties. Information found in the literature regarding chloroprene toxic properties was obtained from studies made with high concentrations of this substance. Results of studies conducted under industrial conditions showed that chloroprene possessed narcotic properties which caused temporary depression of the central nervous system, induced pathological changes in the cardiovascular and respiratory systems, in the gastrointestinal tract, and in the skin. According to E. N. Levina, chloroprene acted as a depilatory. Results of her studies with 2-6 mg/li chloroprene concentrations under acute experimental conditions caused sharp irritation of the throat and eyes; its immediate effect was to produce a temporary intitial sensation of a bitter taste.

A prolonged study conducted by V. G. Mkhitaryan with air containing 4-8 mg/li of chloroprene showed that inhalation of the poisonous vapor in the above concentration for 2-4 hours for 60 days, or longer, lowered the ascorbic acid concentration, the rate of adrenalin flow, reduced the glutathiene concentration, increased the concentration of oxidized glutathione and of the thiolic enzymes, the blood and organ sulfhydryl groups, etc. Such results led to the conclusion that the conversion of the initial substance to the peroxide form played an important part in the development of the above described toxicological picture. The present author compared the toxic picture developed by chloroprene with the clinical picture of radiation disease. The fact that thiolic substances can be used in the therapy of the two conditions supports such an assumption. Results of previously conducted experiments indicated that the threshold of chloroprene odor perception average was 0.9 mg/m^3, and for most odor sensitive persons 0.4 mg/m^3. Chloroprene effect on the functional state of the optical analyzer determined by the method of dark adaptation coincided with the threshold of chloroprene odor perception. On the basis of the above data it was recommended that the maximal single chloroprene concentration in atmospheric air should be 0.25 mg/m^3. At the same time the Committee resolved to adopt 0.08 mg/m^3 as the average allowable maximal 24 hour chloroprene concentration in atmospheric air pending future check determination. The 0.08 mg/m^3 concentration is 1/3 the value of the above recommended maximal single concentration.

In 1960 the present author studied the effect of low chloroprene concentration on the motor nutritional conditioned reflexes of young rats weighing 60 - 90 g, using the now well known Kotlyarevskii chamber. Three conditioned reflexes were established in each rat, two of which were positive in response to ringing of a bell and to the flash of red light, and one negative in response of the sound of a buzzer. These reflexes were grouped into the following stereotypes; two positive in response to bell ringing, two positive in response to flash of red light, one positive in response to sound of bell ringing, one negative in response to sound of buzzer, two positive in response to flash of red light. After the stereotypes were well established (fixed) studies were conducted for the determination of the type of rats' higher nervous activity. The rats manifested the following types of higher nervous activity: a firmly established strong type in rat No. 9, an incompletely balanced strong type in rats Nos. 5, 7, 8, 11, 12, and 13; weak type in rats Nos. 1, 4, and 6. Accordingly, most of the rats' types of higher nervous activity belonged to the poorly or incompletely balanced type, but the degree of the, so-called, nervous instability differed considerably in the different specimens. For example: the degree of nervous imbalance in rats 5, 7, 8, and 11 was manifested sharply, in rats 12 and 13 - much weaker, and in rat 12 instability was slight approaching the well expressed type of higher nervous activity.

Rats Nos. 5 and 13 of the highly imbalanced type, and rats 1 and 4 of the weak type were selected for the experimental inhalation of air containing high concentrations of chloroprene vapor. Rats Nos. 7 and 8 of the imbalanced type, rat No. 9 of strongly balanced type, and rat No. 6 of weak type were exposed to the inhalation of lower chloroprene concentration in the air. The control group contained two rats - No. 11 of strong imbalanced type, and rat No. 12 of the strong type having a weakly manifested imbalance tendency. Rats were exposed to the inhalation of air containing known chloroprene concentrations in two 100 ml capacity experimental chambers. The chloroprene - air mixture was drawn into the chambers and kept evenly mixed by a rotary electric blow fan. The air was filtered through a layer of cotton, silica gel and activated charcoal. Chloroprene concentrations were kept constant by a method previously described for other similar experiments and concentrations were checked at given intervals through the day. Rats inhaled the chloroprene-air mixture 5 hours daily for six continuous months, holidays excluded. Number of inhalation days varied between 153 and 160 days. The chloroprene concentration in chamber No. 1 averaged 3.06 mg/m^3, and in the second chamber 0.56 mg/m^3. All rats were weighed twice weekly, and tested for conditioned reflex activity every other day.

Four of the eight experimental rats showed no symptoms of any disease as a result of the inhalation experiments, rats Nos. 7, 1, and 13 developed pulmonary inflammation; rats 1 and 13 which inhaled higher chloroprene concentration, died during the fifth experimental month. Rat No. 8 died on the sixth experimental month in a condtion of extreme emaciation due to developed cancer of the tongue. It must be noted, however, that some rats became sick even during the pre-experimental observation period. For example, during the period of conditioned reflex training, 14 rats developed pulmonary inflammation and neoplasm formation, rats Nos. 6, 9, 5, and 4 remained free from any sickness during the entire period of inhaling air containing chloroprene vapor; they gained weight at a rate below that of the control rats; under the conditions of the experiment it was safe to assume that this was due to the inhalation of the chloroprene in the air.

Rats of both groups exposed to the inhalation of air containing chloroprene vapor in the two tested concentrations under conditions of chronic experiment suffered functional shifts in their higher nervous system activity at the beginning of the second, third and fourth month of inhalation exposure; records of such shifts appeared as wavy curves. Such changes in the higher nervous system activity appeared as gradual enhancement in the inhibition processes and as weakening in the stimulation processes. This was expressed in a reduced number of intersignal impulses and in the percent of differentiation disinhibition, particularly in rats of sharply defined imbalanced nervous pattern. Thus, in rat No. 5, which manifested 75% of differentiation disinhibition, the percent of disinhibition rose to 82% during the first 42 days of experimental inhalation of 3.06 mg/m^3 of chloroprene vapor, fell to 70% between the 45th and 84th day, and 36% after the 86th day of exposure to the experimental inhalation, as illustrated by blocks in Figure 1. Prior to experimental inhalation exposure differentiation disinhibition in rat No. 7 amounted to 69%. On the 43rd day of chloroprene inhalation in 0.56 mg/m^3 concentration dropped to 55% and between the 43rd and 86th days it dropped to 27%. At the same time, in control rat No. 11, which had a well defined imbalanced neuro-type, differentiation-dis inhibition persisted at the level of 79 - 65%. In rats with a less clearly defined imbalanced type the shifts were not as significant. In rat No. 13 the differentiation disinhibition break-down occurred in 48% of the tests before exposure to inhalation of chloroprene air, and the course of the first 52 days of vapor inhalation it rose to 57% and between the 54th and 102nd days of exposure it dropped to 33%, especially in rats which inhaled air containing 3.06 mg/m^3 of chloroprene. Finally, in rat No. 9 the neurotype was of a well balanced character; differentiation disinhibition occurred only rarely before and during the period of experimental air-chloroprene inhalation; at the same time, other shifts in the central nervous system activity began to appear on the 2nd month of experimental air chloroprene inhalation. More will be said about this later. Differentiation disinhibition appeared in this rat only after 98 days of exposure to the chloroprene vapor inhalation paralleled an ultra paradoxical phase of higher nervous system activity.

Records made of the conditioned reflex activity states in white rats chronically exposed to the inhalation of 0.56 and 3.06 mg/m^3 of chloroprene indicated, as shown in Figure 2 a relatively frequent reappearance of the phase conditions, that is, if equalizing and paradoxical phases.

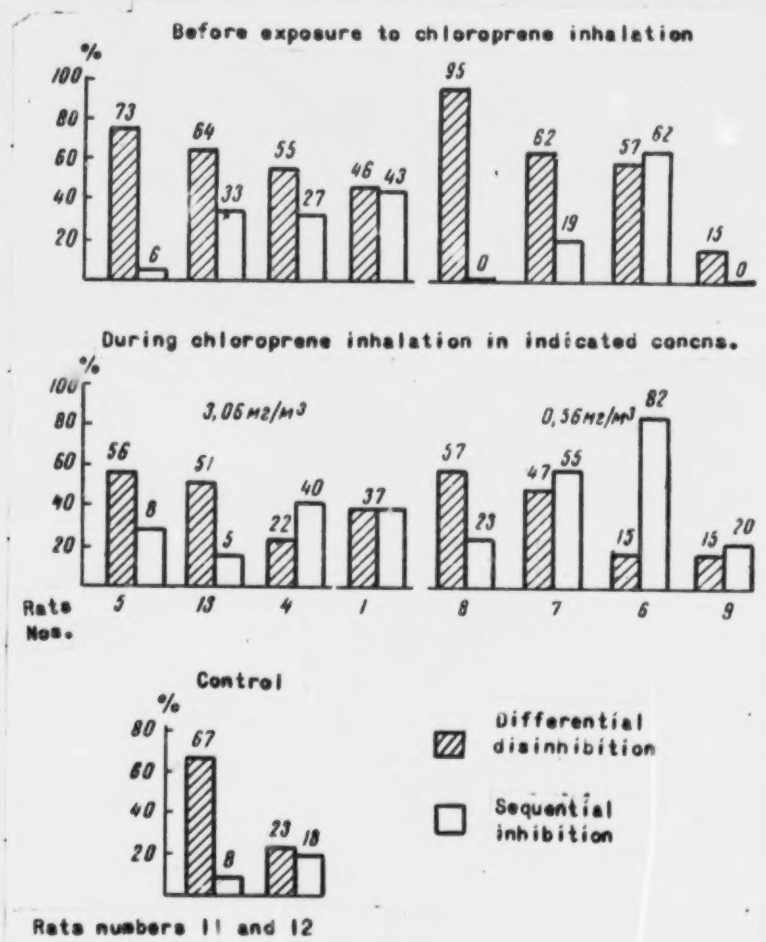

Fig. 1. Manifestation of differential disinhibition and sequential inhibition in the rats

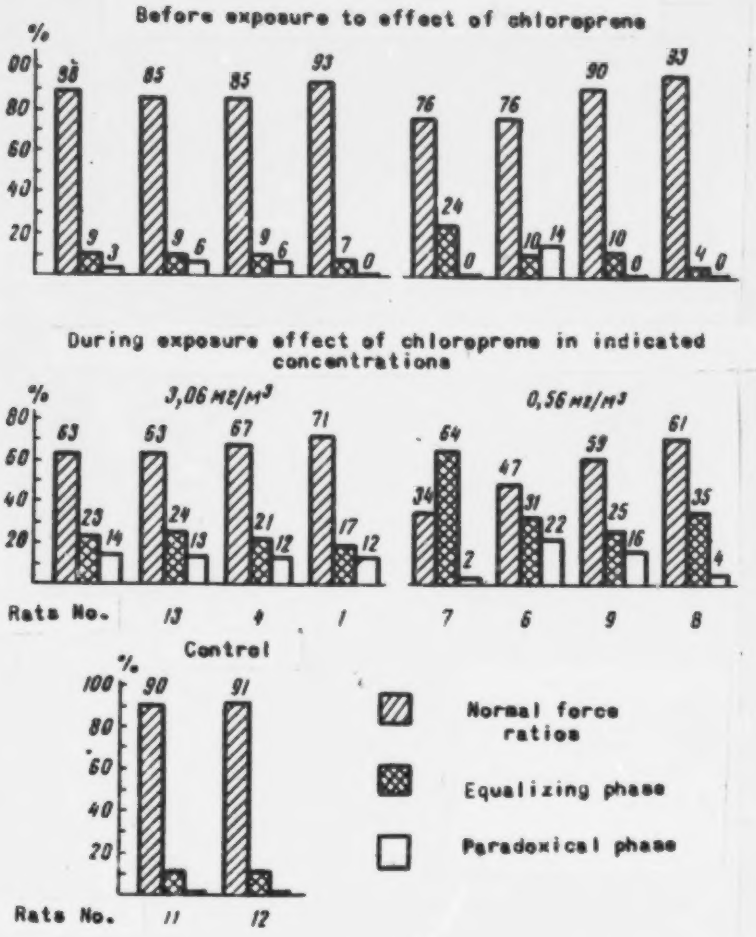

Fig. 2. Force ratios in brain cortex of rats.

Thus, rats of all groups manifested the equalizing phase on the average in 10% of the tests, and the paradoxical phase in 3% of the tests prior to initiating the experimental air chloroprene inhalation. Compared with this, rats which inhaled air containing 0.56 mg/m^3 manifested the equalizing phase on the average in 38% of the tests, and the paradoxical phase in 11% of the tests. In rats which inhaled air containing 3.06% mg/m^3 of the chloroprene the equalizing phase occurred on the average in 22% of the tests, and the paradoxical phase in 13% of the tests.

Inhalation of air containing chloroprene also enhanced the number of instances of positive conditioned reflex fallouts in response to bell ringing and the flash of a red light, as shown by blocks in Figure 3. Data presented on the fallout of positive conditioned reflexes also point to a well defined individual sensitivity on the part of the test rats to the substance under investigation. Rat No. 13 of group 1 retained the same level of conditioned reflex fallouts in response to bell ringing and the flash of a red light all through the experimental period; at the same time the frequency of conditioned reflex fallout in response to bell ringing and flashing of red light

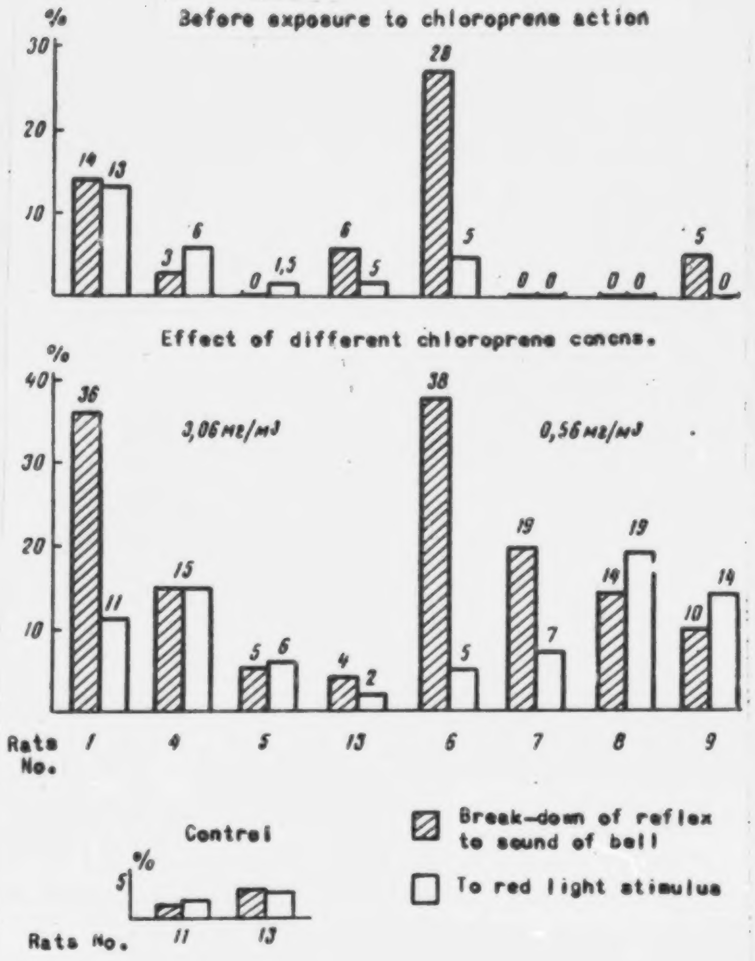

Fig. 3. Falling out of positive reflexes in rats.

increased 4 to 5 fold in the remaining 3 rats of the group. No falling out of positive conditioned reflexes prior to inhalation exposure were noted in 3 rats of group 2; inhalation of air-chloroprene mixture brought about a change in the picture so that 10-19 of the test showed conditioned reflex fallout in response to ringing a bell and 7-19% of fallout in the conditioned reflexes in response to flash of a red light. Such changes in the higher nervous system activity brought about by chloroprene inhalations differed in their intensity depending upon the typological characteristics and the individual sensitivity of rats to the effects of chloroprene. Fifteen to thirty days after inhalation exposure was discontinued the conditioned reflex activity of the experimental rats returned to their initial levels.

Histologic studies of the brain cortex and of several subcortical sections such as cornu ammonis, corpora quadrigemina, thalamus opticus, and pons varolii of rats belonging to two groups, exposed to six months of chloroprene vapor inhalation, showed dystrophy of the nerve cell structures and other morphological changes, as illustrated in Figure 4, and Figures 5 and 6. Indications of regression changes were noted in the walls of the capillaries of the brain cortex and other sections of the brain which may have resulted from the developed vascular wall hypotonia. Many other histopathological changes were noted in the different anatomical brain structures. No such changes were observed in the control animals.

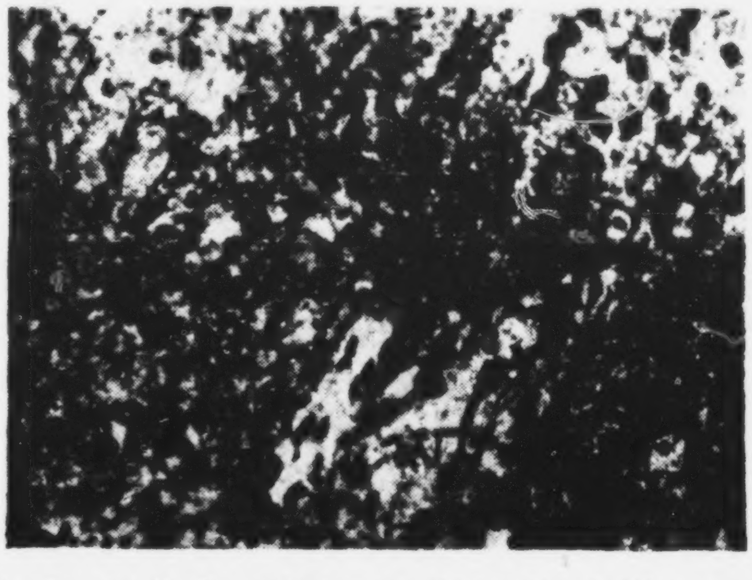

Fig. 4. Rat No. 4. Wrinkled pyramidal cells of fifth layer with clearly manifested protruding terminal branching. (Bil'shovskii impregnation)
Rat No. 5, chloroprene concn. 3.06 mg/m^3

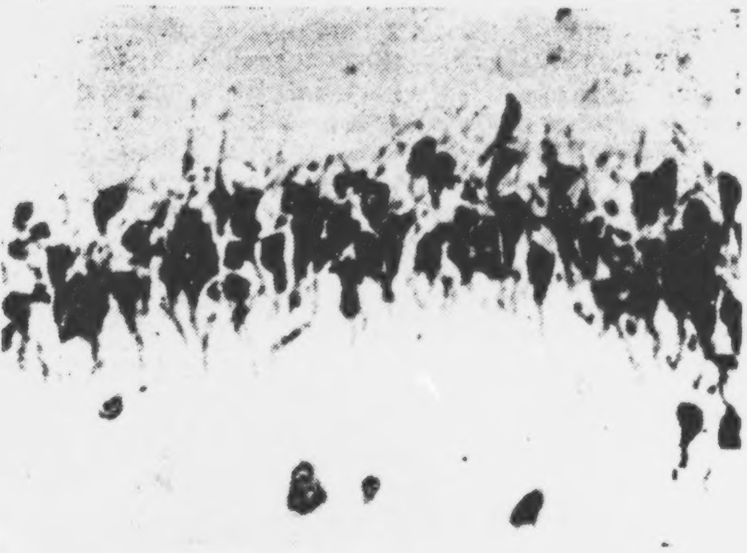

Fig. 5. Swelling and tigrolysis of pyramidal cells.
(Nissle stain)
Rat No. 7, 0.56 mg/m^3 of chloroprene

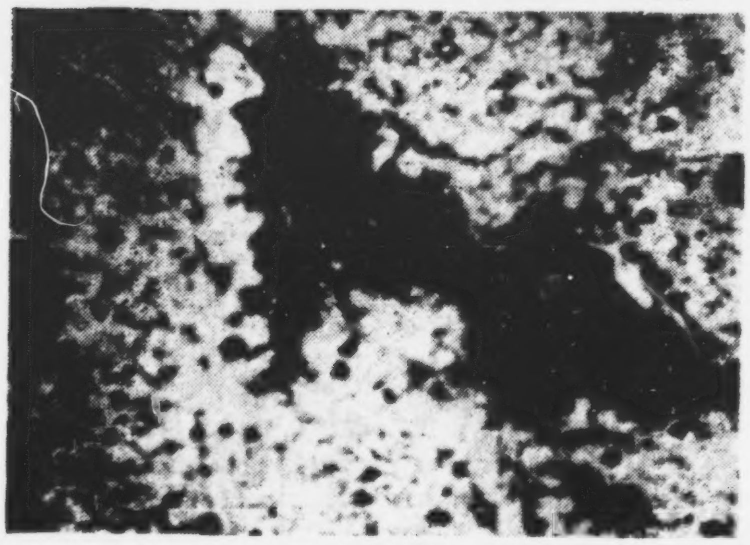

Fig.6. Spheroid cells with breaking down cytoplasm; other swollen cells with branching processes; manifestations of swelling and break-down
(Nissle stain)
Rat No. 9, chloroprene concn. 0.56 mg/m^3

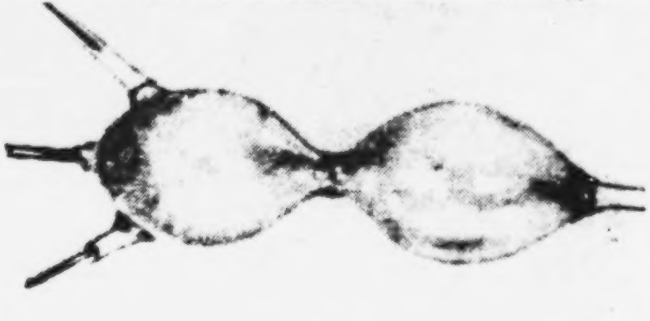

Fig. 7. Upper view of chloroprene mixer and dose delivery apparatus used in 60 days, 24 hour experiments

The present author conducted experiments with 80 young white male rats exposed to the inhalation of chloroprene-air mixtures 24 hours daily for 60 days. The rats were placed into specially constructed exposure chambers of 100 li capacity. Air was supplied continuously by a pressure pump at a regulated rate of 25 li/min. Constancy of the air - chloroprene vapor mixture was maintained with the aid of a mixer, such as is illustrated in Figure 7. The three air outflow extension tubes were 0.5, 0.34, and 0.22 cm in diameter, through which air containing correspondingly 0.5, 0.25 and 0.1 mg/m^3 of the chloroprene was entering into corresponding exposure chambers. Average concentration of chloroprene in the air of chamber 1 was 0.48 ± 0.02 mg/m^3 with a variation in the range of 0.15, 0.85 mg/m^3 in the course of 60 days; concentration of chloroprene in the second chamber was 0.22 ± 0.09 mg/m^3 fluctuating in the range of 0.12 - 0.37 mg/m^3; chloroprene concentration in the third chamber was 0.088 ± 0.004 mg/m^3 fluctuating within the range of 0.05 - 0.19 mg/m^3. The fourth chamber, which housed 20 rats belonging to the control group, received clean air. Rats were kept under experimental inhalation conditions 22 - 23 hours daily for 62 days. Experimental rats were fed twice daily inside the inhalation exposure chamber. At such time the supply of chloroprene vapor and air mixtures was discontinued for 30 minutes. Experimental rats soon became adapted to the experimental conditions and consumed their rations rapidly and with appetite. The rations consisted of ground boiled beef, milk, bread, bran, vegetables, etc.

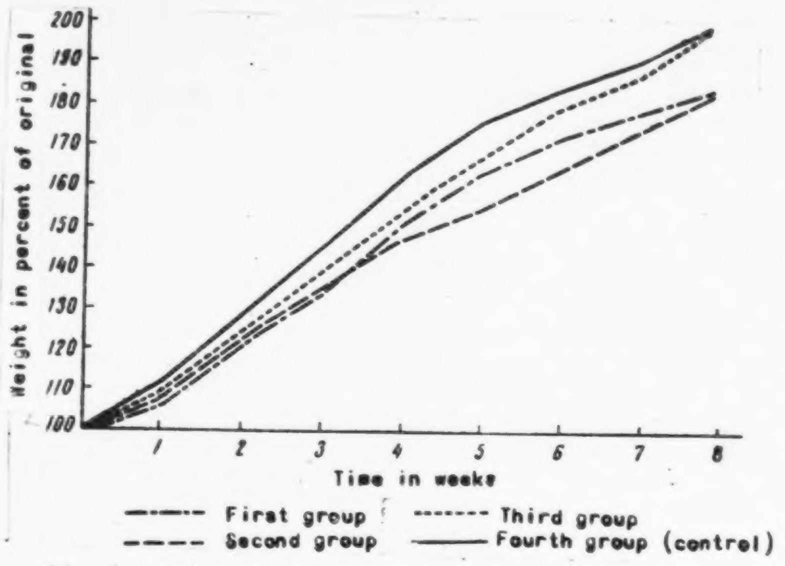

Fig. 8. Body weight changes in rats of different groups.

All rats were weighed daily, records were kept of the amount of water intake; determinations were made of total blood protein, coproporphyrin elimination with the urine, content of vitamin C, total amount of voided urine, vitamin C eliminated with the urine, and of changes in blood cholinesterase activity. Forty of the eighty rats had been sacrificed at the end of 60 days experimental inhalation exposure and the remaining forty were sacrificed two weeks later. Determinations were then made of adenosinetriphosphatase liver activity, the number of brain and liver sulfhydryl groups and weight of the adrenals. All 80 rats persisted in a good state of health, showing no signs of diseases in the course of the experiment. Behavior of the animals showed no deviations from the normal. Analysis of results obtained with weights of the experimental rats showed that beginning with the first inhalation exposure week rats of group

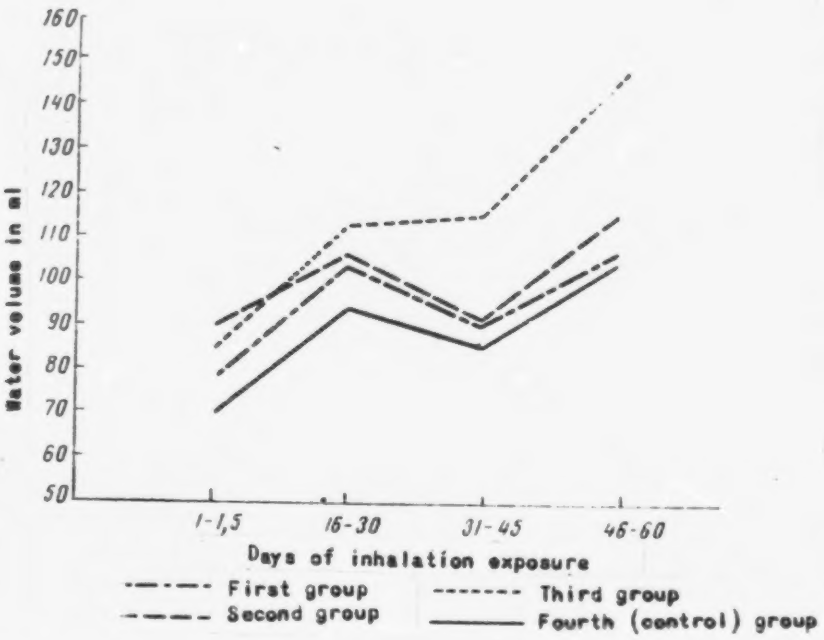

Fig. 9. Changes in volume of daily water intake by rats of the different groups

1, 2 and 3 gained weight at a lowered rate as compared with rats of the fourth or control group. Data thus obtained were of a statistically reliable character since P = 0.01 - 0.05. However, the effect of chloroprene on the rate of weight gain by the rats gradually abated during the second month of experimental inhalation, and finally became equal to the rate of weight gain in the control rats, so that at the end of 8 weeks inhalation, the average gain in weight by rats belonging to group 3 was equal to that of rats belonging to the control group; at the end of the 8th week of experimental inhalation exposure the rate of gain in weight in rats of group 3 amounted to 102.4 ± 2.8 g, as compared with the control gain of 101.5 ± 3.5 g. However, rats belonging to groups 1 and 2 still showed a notably reduced rate in gain of body weight at the end of the 8th week, which in rats of group 1 amounted to 85.9 ±9.4%, and in rats of group 2 amounted to 84 ± 5.9%. This is illustrated by curves presented in Figure 8.

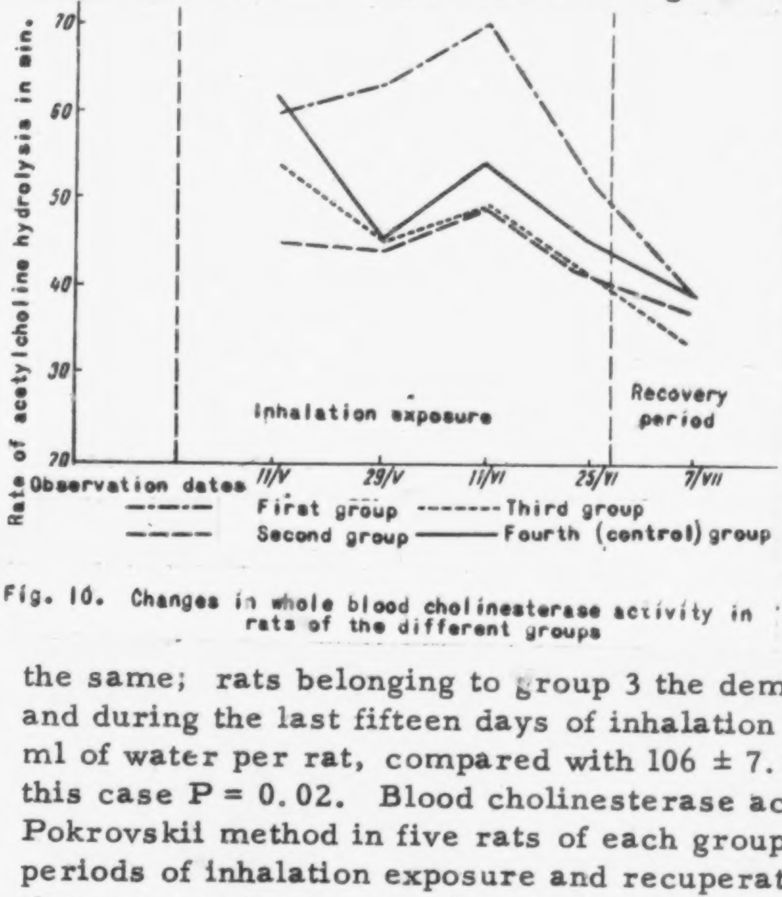

Fig. 10. Changes in whole blood cholinesterase activity in rats of the different groups

It was noted on the 5th - 6th day of inhalation exposure that rats which inhaled chloroprene vapor consumed greater amounts of water than did rats of the control group, and that rats which inhaled air containing lower chloroprene vapor concentrations ingested greater quantities of water, especially as the experimental procedure progressed. Average quantities of water ingested by rats of groups 1 and 2 and the control group daily during two weeks of exposure were approximately the same; rats belonging to group 3 the demand for water gradually increased and during the last fifteen days of inhalation exposure amounted to 147 ± 13.1 ml of water per rat, compared with 106 ± 7.9 ml for the control group; in this case P = 0.02. Blood cholinesterase activity was determined by the Pokrovskii method in five rats of each group once fortnightly during the periods of inhalation exposure and recuperation. The enzyme hydrolysis time was on the average 63 minutes in rats of the first group at the end of 30 days inhalation exposure. In other words it was 1.4 times greater than in rats of the control group. After 90 days inhalation exposure the enzyme hydrolysis time was 69 minutes, which dropped to 51 minutes at the end of the experimental inhalation exposure, and to 38 minutes at the end of the recuperation period. This is illustrated by curves in Figure 10. Cholinesterase activity rose to a statistically unverified level in rats of groups 2 and 3 during the entire period of exposure and recuperation, which can be regarded as a mere tendency to loss in the hydrolytic intensity of the enzyme activity. It was clearly indicated that susceptibility to the effect of chloroprene vapor greatly differed with individual rat specimens. Therefore, it was thought desirable to analyze results obtained with individual rat specimens. In 4 of the 5 tested rats of group 1 the drop in cholinesterase activity was significant; in 2 of the rats the maximal drop was noted at the end of 30 days, and in the other 2 at the end of 90 days of experimental inhalation, after which rate of cholinesterase hydrolysis began to rise, returning to its normal level at the end of the recuperation period.

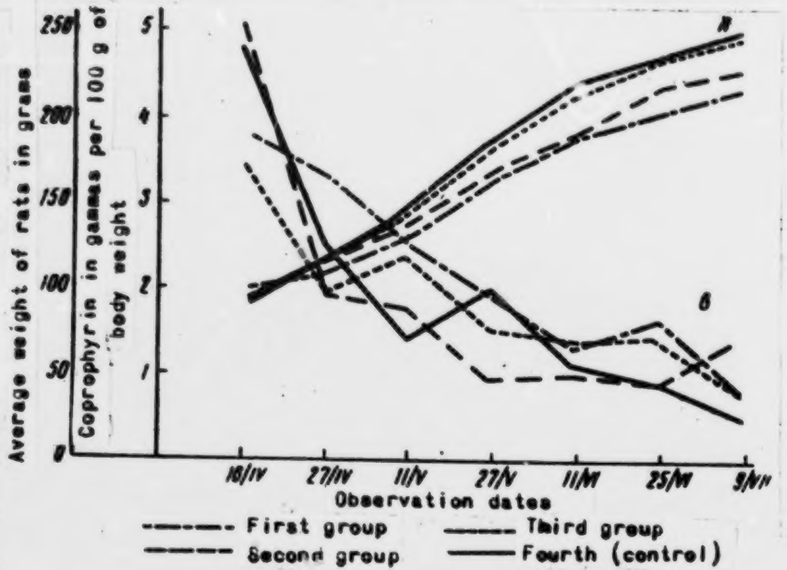

Fig. 11. Rate oc coproporphyrin elimination *via* the urine (B) and animal body weight (A)

No deviations of any kind were noted in one of 5 rats belonging to group 1 through the entire period of experimental inhalation. All 5 rats belonging to group 2 showed a negligible rise in the rate of blood cholinesterase activity at different periods of the experiment; this occurred in rat No. 16 at the end of 30 days, in rats 17, 18 and 20 at the end of 45 days, and in rat No. 19 at the end of 60 days. Rat. No. 19, belonging to group 3, showed no deviation from the normal. Rat No. 16 showed considerable drop in cholinesterase activity at the end of 30 days. Similar changes in blood cholinesterase activity had been noted in other experimental rats. Accordingly, analysis of average data and of data obtained for individual rats, indicated that 0.48 mg/m^3 of chloroprene constituted the threshold effect of this vapor on blood cholinesterase activity, and that it effected a drop in the activity of this enzyme, which, however, became less effective at the end of the experimental inhalation period.

Determination of coproporphyrin elimination rate with the urine was determined by the modified Fisher method. Determination was conducted with 24 hour urine collected from 5 rats belonging to each of the four groups for 15 days. Curves in Figure 11, record changes in grams of coproporphyrin per 100 grams of average rat body weight, according to groups. The curves show that reduction in coproporphyrin elimination rate with the urine followed a wavy course, and that it varied with the age of individual rats. In rats belonging to group 1 rate of coproporphyrin elimination with the urine slightly increased; in rats of group 2 the increase was less pronounced, and in rats of group 3 was of an intermediate character. However, all such observations proved to be statistically insignificant. Therefore it was concluded that the effect of chloroprene on prophyrin metabolism was below its threshold effect.

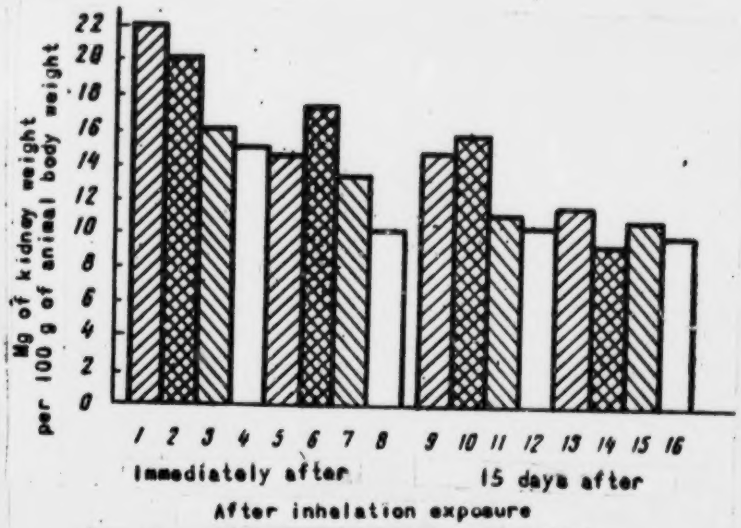

Fig. 12. White rats' kidney weight
1, 5, 9, 13 - rats of first group; 2, 6, 10, 14 - rats of second group; 3, 7, 11, 15 - rats of group three; 4, 5, 12, 16 - rats of group four (control) group

Blood protein fractions were determined in five rats of each of the three experimental groups through the inhalation period on the 19th - 22nd, 38th - 41st, and 54th - 57th date of exposure by the electrophoretic paper partitioning method, followed by staining with bromphenol blue. Electrophoregrams were processed as described elsewhere. Record from all groups fluctuated in the concentration of individual blood protein fractions, especially in the globulins. On the 20th-40th inhalation day albumins constituted 40 - 55% of blood serum fractions in rats of all groups with no noteworthy differences between the groups. On the 54th - 57th inhalation day the blood albumin per cent of rats belonging to group 1 was 52.3 ± 3.2; in rats of group 2 it was 36.7 ± 6.6; in rats belonging to group 3 it was 46.3 ± 6.8, and in rats of the control group it was 49.2 ± 5.8. In rats of group 3 the albumin globulin ratio was practically the same as in rats of the control group. In rats of group 2 a slight shift was noted in the blood proteins in the direction of globulins, and in rats belonging to group 1 there was a negligible shift in the direction of albumins.

The content of vitamin C in 24 hour urine of the rats was determined by the Tilman reagent. Twenty-four hour urine specimens were collected in specially constructed containers. Vitamin C in the urine was determined every 15 days. Results indicated that 0.011 - 0.48 mg/m^3 of chloroprene inhaled by rats 24 hours daily for 60 days had no effect on the amount of vitamin C eliminated *via* the urine. Average vitamin C concentration in the urine of rats belonging to group 1 was 0.018 mg/ml, in group 2 0.015 mg/ml, and in rats belonging to group 3 it was 0.016 mg/ml, as compared with 0.015 mg/ml in rats of the control group. Determinations were also made of vitamin C in the urine of rats which had been administered an average of 0.5 mg of the vitamin. Results indicated that on the 16th day there was no vitamin deficiency in the experimental animals, and that on the 32nd and 47th day of experimental inhalation of the toxic vapor vitamin C was eliminated *via* the urine by rats belonging to groups 1, 2 and 3. However, rats inhaling chloroprene vapor for 61 days which received 0.5 mg of vitamin C per day manifested no vitamin C deficiency in the organism. The amount of vitamin C eliminated in 24 hours via the urine of rats belonging to group 1 was 0.34, of rats belonging to group 2 0.31, of rats belonging to group 3 0.33, and of rats belonging to the control group it was 0.35 mg.

Weight of adrenals was determined in 40 rats sacrificed after 60 days of continued exposure to chloroprene inhalation, the same was done with the adrenals of rats sacrificed 15 days after discontinuation of chloroprene inhalation, and the effect of the poisonous vapor on the adrenal function was recorded. Results are shown in block form in Figure 12. Rats of groups 1, 2 and 3, which had been sacrificed at the end of the experimental inhalation period, showed a rise in the average adrenal weight as compared with adrenals of rats belonging to the control group. Thus, the average adrenal weight in mg % of rat weight of the control croup was 15.58 ± 0.05 as compared with 22.08 ± 3.22 in rats of group 1 and 20.79 ± 3.21 of rats belonging to group 2 and 16.92 ± 0.33 of rats belonging to group 3. In other words, adrenals of rats belonging to groups 1 and 2 increased by 25% as compared with the adrenals of the control group; adrenals of rats belonging to group 3 increased only by 3% as compared with those of the control group. It should also be noted that fluctuation values in the weights of adrenals of individual rats were of the negligible order in the control and 3rd group, and that in individual rats belonging to groups 1 and 2 such fluctuations ranged between 29.2 and 33.8% of the weight of rats body weight. This indicates that chloroprene effect on the adrenal function was a negligible one. Rats belonging to group 1, which were sacrificed at the same time, showed analogous shifts in the adrenal functions. The average adrenal weight in such rats was 10.82 ± 0.6 in the control group, 14.64 ± 0.41 in rats belonging to group 1, 17.50 ± 1, 71 in rats belonging to group 2, and 13.10 ± 0.99 mg per 100 g of average rat body weight in rats of group 3. At the end of the recuperation period weight of adrenals of rats belonging to group 4 were in close correspondence amounting from average to 11.52, 9.30, 10.90, and 10.07 mg per 100 grams of average rat body weight. In rats belonging to groups 1 and 2 the difference compared to rats of the control group was considerable; no such difference was noted in the adrenals of rats be-

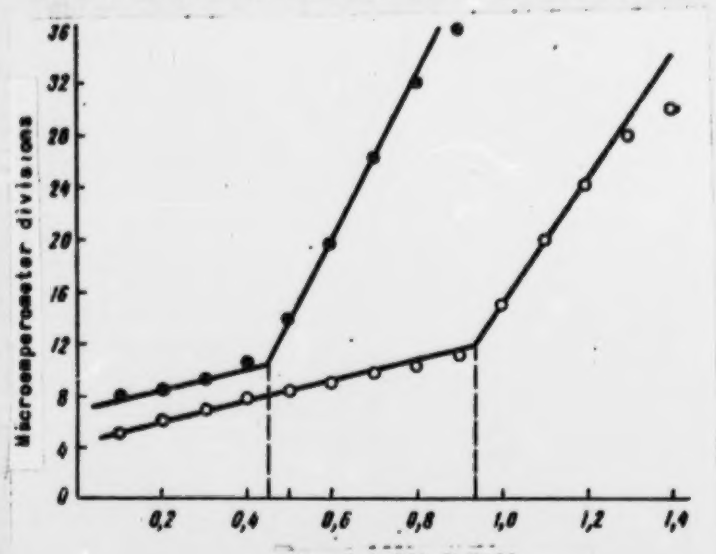

Fig. 13. Results of $HgCl_2$ amperometric titration of cysteine

longing to group 3. The average weight of adrenals in mg per 100 g of rats body weight was correspondingly as follows: 14.62 ± 1.28, 15.56 ± 2.26, 11.14 ± 0.88, and 10.48 ± 0.65. Accordingly 0.22 mg/m^3 of chloroprene constituted the threshold concentration of effect on the adrenal function of white rats, and 0.088 mg/m^3 constituted the subthreshold effect concentration.

Sulfhydryl groups in brain and liver homogenates were determined by the amperometric mercuric chloride titration method after rats had been exposed to the inhalation of air containing chloroprene vapor 24 hours daily for 60 days; 10 rats of each group were used for the brain sulfhydryl group determination, and 5 rats of each group for the determination of mixed brain and liver homogenates sulfhydryl groups. Homogenates were prepared and sulfhydryl groups determined at low temperature. Titration was conducted with 0.001 N mercuric chloride solution. Results of titrations conducted with 0.001 N solution of cysteine under similar conditions showed that the number of sulfhydryl groups found in the organs closely approximated the number of true sulfhydryl groups in the organs, as shown by curves presented in Figure 13. Curves in Figure 14 present results of brain homogenates titration of 2 rats. The results point to a range of 9.5 - 11.5% statistically significant lowered numbers of sulhydryl groups in brain homogeneate of rats belonging to groups 1, 2, and 3 after exposure to the inhalation of chloroprene-air mixture 24 hours daily for 60 days For example, the sulfhydryl groups in the brain homogenate of rats belonging to the control group averaged 1.65 mg per 100 g of fresh tissue, which fell to an average range of 1.46-1.50 mg per 100 g of fresh tissue in rats which experimentally inhaled air containing given concentrations of cholorprene vapor, as illustrated by blocks in Figure 15. V. G. Mkhitaryan exposed rats to the inhalation of air containing 8 mg/li of chloroprene vapor 2 hours daily for 30 days and found that the number of sulfyhdryl groups in the brain tissue fell to 48.2% of the controls. During the recuperation period the number of sulfhydryl groups in the brain tissue gradually increased, returning to the original level in all experimental rats at the end of the recuperation period. Results of statistical analysis indicated that any apparent differences were of a negligible character.

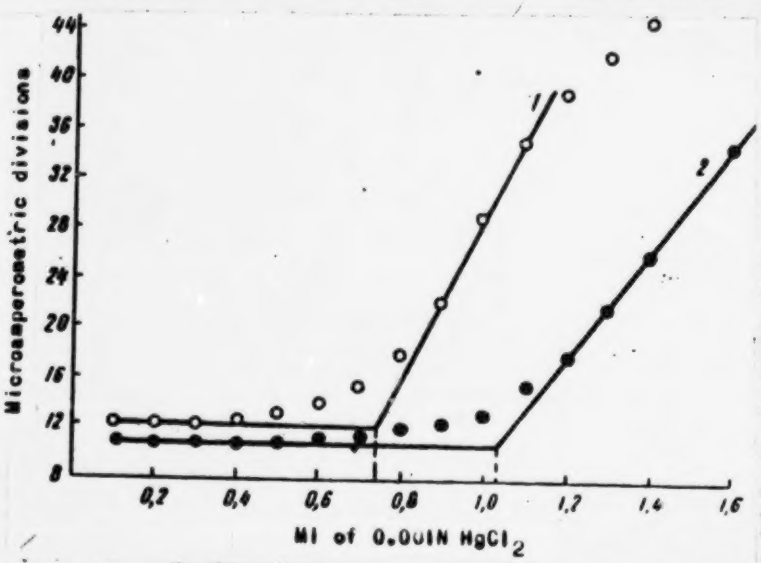

Fig. 14. Sample of amperometric titration of sulfhydryl group of rats' brain

1 - Rat No 3 of First group; 2 - Rat no. 2 of Control group

The number of sulfyhdryl groups in brain homogenate of rats belonging to group 1 was by 4.2% below that in rats of the control group; in the brain homogenate of rats belonging to rats of group 2 the number of sulfhydryl groups was the same as in the control rats, and in rats belonging to group 3 the number of sulfhydryl groups exceeded the number of sulfhydryl groups in the brain homogenates of individual rats belonging to one and the same groups fluctuated within significant limits. Immediately after the termination of the experimental inhalation period differences among individual rats of the same group were within the range of ± 14 - 17%; at the end of the recuperation period the fluctuation ranged between ± 4 - 10%, and in rats belonging to group 1 the fluctuation range fell to ± 6 - 15%, indicating that chloroprene inhalation by rats belonging to groups 1 and 2 affected the test rats toxicologically, but had no apparent toxic effect on rats belonging to Group 3. The number of sulfhydryl groups in liver homogenates of rats belonging to the three experimental groups exceeded by 10% the number of corresponding SH-groups in liver homogenates of the control group rats. However, absolute values were statistically nonsignificant.

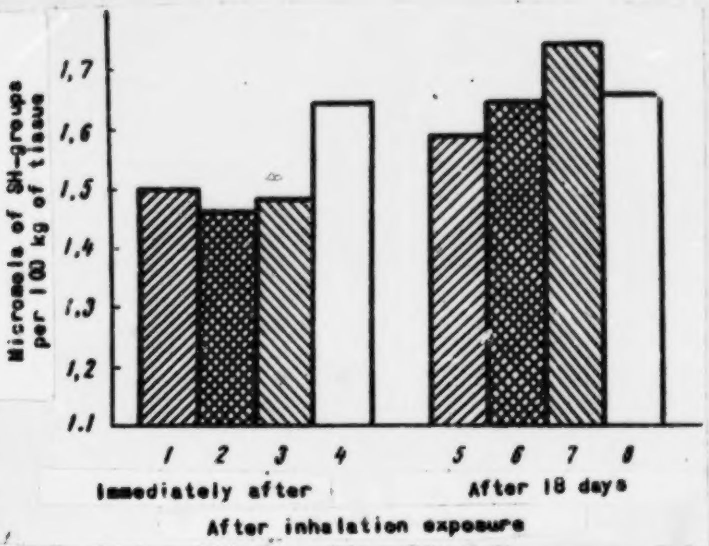

Fig. 15. Sulfhydryl groups in brain homogenate

Fig. 15. Sulfhydryl groups in brain homogenate

1 and 5 - Rats of First Group; 2 and 6 - Rats of Second group; 3 and 7 - Rats of third group; 4 and 8 - Rats of Control group

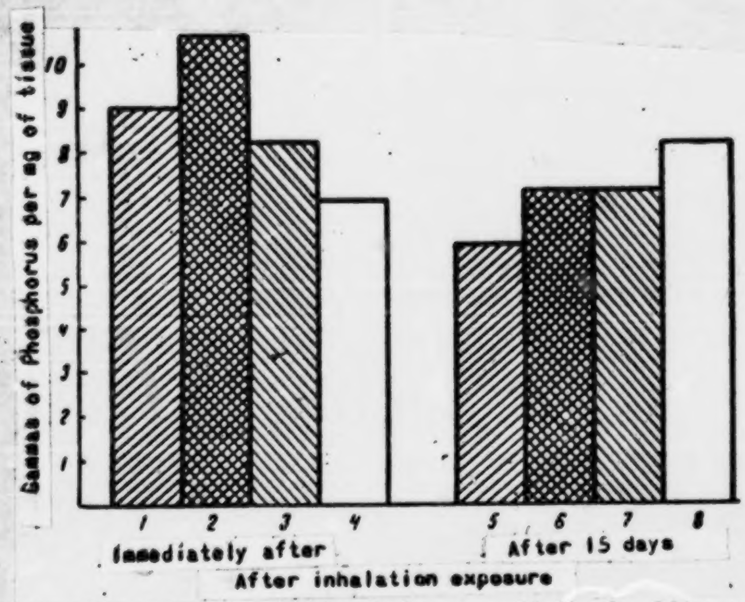

Fig. 16. Adenosine triphosphatase activity in white rats' liver

1 and 5 - Rats of First group; 2 and 6 - Rats of Second group; 3 and 7 - Rats of Third group; 4 and 8 - Rats of Control group

Results of the present investigation indicated that brain tissue sulfhydryl groups were highly sensitive to the action of chloroprene vapor as compared with the sulfhydryl groups of the liver tissue. This was also verified by the fact that value obtained in the reduction of brain tissue sulfhydryl groups were statistically significant, whereas values obtained in the increased liver tissue sulfhydryl groups were not statistically significant. On the other hand, brain tissue, which possessed a greater recuperative vitality, rapidly regenerated its normal number of SH-groups, whereas the increase in number of liver sulfhydryl groups persisted to the end of the 60-day experimental inhalation period.

Activity of liver adenosine triphosphatase activity was determined by Mkhitaryan modification of the Dubois and Potter method based on the determination of inorganic phosphorus by the Fisk and Subboray method. With the aid of this procedure V. G. Mkhitaryan demonstrated that adenosine triphosphatase activity fell to lower levels in organs of white rats inhaling chloroprene vapor.

According to data obtained by this author, the adenosine triphosphatase liver activity of white rats exposed to the inhalation of air containing 8 mg/m^3 of chloroprene vapor 2 hours daily for 90 - 180 days, became reduced by 47.6 - 57.4%. The present author assumed that the fall in the adenosine triphosphatase activity was basically brought about by chloroprene peroxide which oxidized the SH-groups, and also by the resulting C hypovitaminosis. Adenosine triphosphatase liver activity was determined in 20 rats sacrificed by decapitation immediately after the termination of the experimental inhalation period, and 20 rats sacrificed 15 days later. Livers of the sacrificed rats were quickly removed and homogenized at low temperature; 100 mg of the homogenate was made up to 10 ml by the addition of distilled water; 0.1 and 0.2 ml of the liver homogenate were placed into each of the two test tubes using a third test tube for control purposes; 0.15 ml of diethylbarbiturate buffer of pH - 7.4 was added to each of the three test tubes, followed by 0.05 ml of 0.004 M solution, and 0.15 ml of 0.013 M solution of adenosine triphosphatase. Distilled water was then added to each o of the three tubes to bring the volume up to 0.65 ml; the tubes were incubated at 37° for 15 min. The reaction was then arrested by the addition to each tube of 0.1 ml of 50% solution of trichloroacetic acid. The test tubes were then centrifuged at 3000 rpm. The inorganic phosphorus was determined electrocolorimetrically in 0.3 ml of the sediment released by the ATP. Activity of liver adenosine triphosphatase was expressed in γ of inorganic P split off from the ATP in 15 min. per 1 mg of fresh liver tissue.

Results pointed to an increase in adenosine triphosphatase activity elicited by chloroprene inhalation, as illustrated by blocks in Figure 16, which was in agreement with the data indicating an increase in the liver SH-groups. The 30% increase in rats of group 1 as compared with the control group, was statistically verified, but data indicating an approximate 20% rise in rats belonging to Group 3 were not significant statistically. Liver adenosine triphosphatase activity in γ per 100 mg of fresh liver tissue, as determined at the end of the experimental chloroprene vapor inhalation,

Table I

AVERAGE DAILY CHLOROPRENE CONCENTRATIONS IN THE AIR SURROUNDING THE PLANT

Meters from plant	1954			1961		
	Number of Samples	Chloroprene in mg/m³		Number of Samples	Chloroprene in mg/m³	
		Average	Maximal		Average	Maximal
250	10	1,87	3,8			
500	10	1,94	4,0	7	0,19	0,47
1 000	10	1,04	2,4			
2 000	9	0,62	0,99	7	0,13	0,27
3 000				7	0,06	0,19
5 000				10	0,18	0,42
7 000	26	0,15	0,34	10	0,24	0,50

Note: In 1961 air samples were collected at 500 and 700 meters from the plant in the month of February, and at 3000 meters from the plant during the month of April

amounted to 0.04 ± 0.37 in rats of group 1, 10.72 ±1.05 in rats of the second group, 8.36 ± 0.37 in rats of group 3, and 7.00 ±0.65 in rats of the control group. Results obtained 15 days after stopping the experimental chloroprene vapor inhalation confirmed previous results, which indicated that inhalation of air containing 0.088 mg/m³ of chloroprene vapor had no harmfull effect on rats' organism under the experimental conditions, but that 0.48 mg/m³ of chloroprene vapor was highly toxic; inhalation of air containing 0.22 mg/m³ of the vapor was also toxic under the experimental conditions, but less than the 0.48 mg/m³ concentration, as shown by the fact that under such experimental inhalation conditions the liver adenosine triphosphatase activity rose by 25% with p = 0.01. After the 15th recuperation day liver adenosine triphosphatase activity was 6.16 ± 0.60 in rats of Group 1, 7.30 ± 0.75 in rats of Group 2, 7.38 ±1.03 in rats of Group 3, and 8.42 ±0.40 in rats of the control group. Results of studies made of chloroprene vapor effect on the functional activities of rats exposed to the inhalation of air containing chloroprene 24 hours daily for a 60 day period, at different chloroprene vapor concentration showed the following: the 0.088 mg/m³ concentration can be recommended as the maximal 24-hour average concentration in atmospheric air, with 0.088 mg/m³ as the subthreshold concentration. The 0.48 and 0.22 mg/m³ chloroprene concentrations under the experimental conditions elicited unfavorable shifts in some functional systems of the experimental rats.

The pattern and extent of chloroprene air pollution in the proximity of a pertinent industrial plant was studied in 1961. Results brought out that the general situation has improved since the previous investigation conducted in 1954, as shown by data presented in Table 1. Paradoxically as it may seem, this was more in evidence the closer the investigation approached to the source of chloroprene discharge into the atmosphere. The improvement in the atmospheric air pollution, was briefly described above, due to the introduction of more modern methods and processes of manufacture. Therefore, it is recommended that improved methods of production and processing be continued to be introduced more speedily, and in addition, hermetization of ventilation air conduits should be assured and more efficient, and that the plants should be equipped with appropriate discharge air and gas purifying installations without delay.

Conclusions

1. Continuous 6 months experimental inhalation of air containing 3.06 and 0.56 mg/m^3 of chloroprene 5 hours daily brought about functional shifts in the higher nervous activity of experimental rats, as evidenced by the appearance of histologic changes in the neuro-cells of the brain cortex and subcortex and of tigroid substances in the vascular brain system. No visible changes appeared in the synaptic apparate.

2. Results of 6 months exposure of white male rats to the inhalation of chloroprene under the described experimental conditions 5 hours daily were as follows:

a) chloroprene of 0.48 mg/m^3 brought about a reduction of the whole blood cholinesterase activity; chloroprene of 0.22 and 0.088 mg/m^3 elicited in most experimental rats clear evidence of increased blood cholinesterase activity at different period of inhalation confirming the previously noted manifestations regarding the clear-cut differences in individual rats to the animals' sensitivity to the effects of chloroprene.

b) chloroprene of 0.48, 0.22, and 0.088 mg/m^3 had only a subthreshold effect on the functional system of the experimental rats; and

c) at the end of the second month the inhalation of 0.22 mg/m^3 of chloroprene elicited shifts in the blood globulins; this was not noted in experimental rats which inhaled air containing higher chloroprene concentrations. It can be assumed that the action of higher chloroprene concentrations might produce reverse effects, that is, shifts in the blood albumins;

d) chloroprene of 0.48, 0.22, 0.088 mg/m^3 had no effect on vitamin C level in voided urine. Administration to the experimental rats of 0.5 mg of vitamin C per rat brought out the fact that the organs of the experimental rats suffered a vitamin C deficiency beginning with the 30 - 45th day of exposure to inhalation and of complete absence of vitamin C at the end of two days experimental inhalation.

e) chloroprene in 0.48 and 0.22 mg/m^3 unfavorably affected the adrenal function, as shown by the size and weight increase of the gland. 0.088 mg/m^3 of chloroprene vapor increased the weight of adrenals only slightly returning to the original normal weight after two weeks of recuperation.

f) inhalation of air containing 0.22 mg/m^3 of chloroprene lowered the number of SH-groups in the brain tissue. Inhalatinn of air containing 0.088 mg/m^3 of chloroprene likewise lowered the number of SH-groups in the brain tissue. Two weeks after the experimental inhalation was discontinued the number of SH-groups in the brain tissue returned to normal. Brain SH-groups were more sensitive to the effect of chloroprene than were sulfhydryl groups of liver tissue, as shown by the fact that inhalation of air containing 0.088 mg/m^3 of chloroprene had no effect on the SH-groups of liver tissue.

g) the threshold effect of chloroprene should be set at 0.22 mg/m^3. Such a chloroprene concentration elicited an increase in the adenosine triphosphatase activity of experimental rats' liver.

3. Chronic 24 hour daily inhalation of air containing chloroprene in low concentration showed that adrenal functions were most sensitive to the effects of the poisonous vapor; this was followed by the sensitivity of the brain SH-groups and by the activity of liver adenosintriphosphatase.

4. On the basis of above cited results it is believed that the 24 hour maximal average chloroprene concentration in atmospheric air should be set at 0.08 mg/m^3, as recommended by the "Chief Government Sanitary Inspection of the USSR."

5. Atmospheric air studies conducted in the proximity of plants related to chloroprene air pollution led to the conclusion that new and more effective processes of production should be introduced as soon as possible; parallel with this, up-to-date ventilation systems and air and gas purifying equipment should be installed at the earliest possible opportunity.

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TOXICITY OF SULFUR OXIDES IN CHRONIC PULMONARY INSUFFICIENCY (EXPERIMENTAL STUDY)

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Hygienic standardization of atmospheric air pollution takes into consideration primarily the effect of air pollutants on the most susceptible inhabitants, such as children, senior citizens, and persons having chronic diseases. The rationality of such an approach to the problem of air pollution is selfevident. During 1-5 December of 1930 toxic smog, which occurred in the Maass Valley in Belgium, older people, children and persons suffering of respiratory and cardiovascular diseases were affected most, and most of the 60 persons who died during the smog period were individuals of advanced age and patients who had chronic pulmonary and cardiovascular disease. During the 1948 Donora smog in the USA, more than 60% of the affected persons were in the range of 52 and 84 years of age, and 42.1% of the local population suffered of the smog effect. Eighty percent of the smog affected population consisted of persons who had bronchial asthma, chronic bronchitis, and cardiovascular disease. The situation was of an analogous character during the 1952 London smog. Reports found in the literature pointed to the effect of atmospheric air pollution on the frequency and persistence of asthmatic attacks, among persons who had bronchial asthma.

Results presented by several authors on the consequences of smogs were expressed in terms of lost men-days. Thus, at 0.149 mg/m^3 air pollution with oxides of sulfur gas the bronchial men-days index was 0.081, at 0.150 - 0.1350 mg/m^3, the bronchial men-days index was 0.120, and in regions where the air was polluted with 0.350 mg/m^3 or more of sulfurous gas the bronchial men-days index rose to 0.438. The data support the principle of hygienic standarization of pollutants in atmospheric air on the basis of results obtained with most susceptible population members, even though it is felt, that such a basis of hygienic air purity standardization should be suppored by supplemental studies. Especially with regard to the following; 1) do all air pollutants intensify the gravity of existing pulmonary and cardiovascular diseases, or is it due only to sulfurous gas pollutants which according to some investigators, constituted the basic pollution component during the prevalence of toxic smogs; 2) how great must the safety coefficient be in determining MAC of atmospheric air pollutants in the presence of sick or otherwise susceptible residential groups. Naturally, this question can be answered only by conducting experiments under specifically pertinent conditions. In this connection, the present author compared thresholds of lethal concentrations for healthy animals and for animals having chronic pulmonary insufficiency for each member of the sulfurous oxides, individually and in combination. Guinea pigs and white rats were used as a model of chronic pulmonary insufficiency.

Experimental silicosis in white rats has been previously described by many investigators including the procedures by which experimental silicosis has been produced in the laboratory animal; in many instances the etiological agent was administered intratracheally. No experimental silicosis has been previously described by any investigator; however, S. Ya. Barenblat, M. M. Vilenskii, and I. A. Kusevitskii in 1935 produced experimental asbestosis in guinea pigs. The present author used the intratrachaeal method of administering dust to guinea pigs. This procedure produced a clear clinical picture of silicosis within 4 to 6 months of administering 30-75 mg of free silicon dioxide. Five months after the injection of the silicon dioxide dust the experimental guinea pigs and white rats were examined for the presence of silicosis symptoms. Results verified conclusions arrived at in the preliminary investigation. Microscopic lung examination of animals treated with silicon dioxide showed in all instances interstitial pneumonia with incipient sclerosis. There were signs of catarrhal bronchitis with regenerative mucous membrane. Also hyperplasia and incipient sclerosis of the bronchial lymphatic nodes. Signs of emphysema appeared in the normal parts of the lungs, as illustrated in histologic sections presented in Figures 1 and 2, and which are characteristic of stages I - II in experimental silicosis.

Studies with white rats. Tests were conducted with 50 white rats weighing 207.2 ±11 g before the introduction of the silicon dioxide dust. Experimental silicosis appeared in 25 of the rats. The experimental animals had been distributed as follows: 15 rats with experimental silicosis and 15 rats free from silicosis received intratrachaeally; 10 rats with silicosis and another 10 rats free from experimental silicosis were kept as controls. Each cage housed 5 rats with experimental silicosis and 5 rats free from silicosis used as controls. Rats were exposed to silicon dioxide dust inhalation for 8 hours. Experimental animals housed in chamber 1 were exposed to the action of sulfurous acid vapor of 1,000 mg/m^3; animals housed in chamber No. 2 were exposed to the effect of sulfuric acid aerosol in 300 mg/m^3 concentration; animals housed in chamber No. 3 were exposed to the inhalation of combined sulfurous acid aerosol and sulfuric acid at same concentrations, selected on the basis of data found in the literature regarding the effect of sulfur oxides.

Some authors found that the absolute lethal sulfuric acid aerosol concentration for white rats was in the range of 770 - 800 mg/m^3, and the LD$_{50}$ within the range of 450 - 540 mg/m^3. No data were found in the literature regarding the lethal concentration of sulfurous acid to white rats. However, evidence was found that 1500 mg/m^3 acting for 6 hours produced no deaths among experimental animals. Actual sulfur oxide concentrations in the exposed chambers were as follows: sulfurous gas - 965 ± 97.0 mg/m^3; sulfuric acid aerosol - 304 ± 27.0 mg/m^3; combination of sulfuric acid and sulfuric acid aerosol - 977.5 ± 72.8 mg/m^3, and 303.5 ±29.6 mg/m^3 correspondingly. Thus, sulfuric acid aerosol was selected in concentrations below the LD$_{50}$, amounting to 0.38 of the first and 0.61 of the second concentration. The sulfuric acid concentration amounted to 2/3 of the tolerance concentration to the animal species used in the experiments. Results are listed in Table 1. Data presented in Table 1 show that the effect of each oxide of of sulfur individually produced no deaths among the animals in the course of 7 days inhalation exposure, or at any time after the experimental inhalation was discontinued. This was equally true of animals

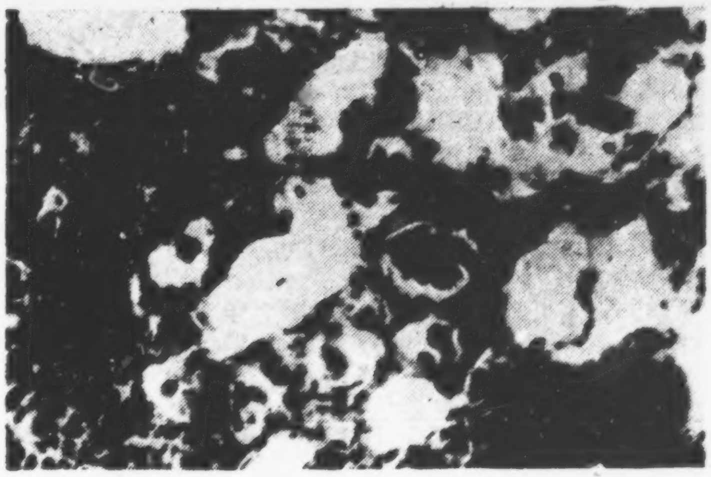

Fig. 1. Lung of guinea pig 6 months after administration of 50 mg. of silicon dioxide

Desquamative bronchopneumonia. Cell conglomerates in alveolar lumens

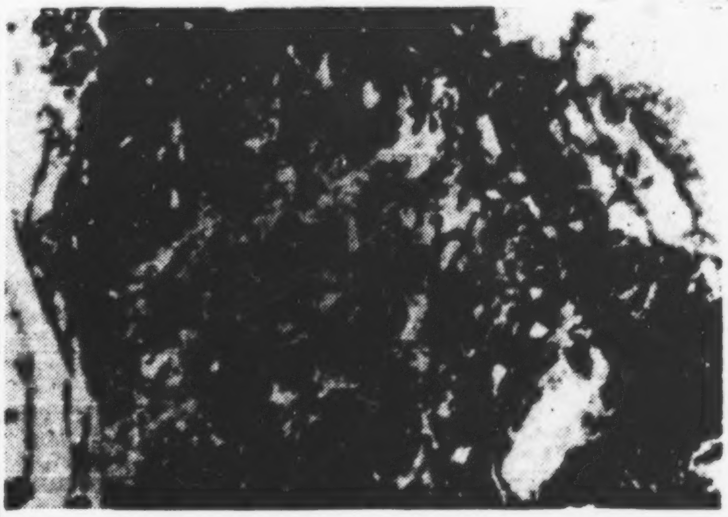

Fig. 2. Rat lung 6 months after administration of 50 mg. of silicon dioxide

Silicon nodule

Table I

EFFECT OF SULFUR OXIDES ON WHITE RATS :

Concentration in mg/m3	Number of animals		Number of animals	
	Number of silicosis-positive	Number died	Unaffected	Died
SO_2—965	5	—	5	—
H_2SO_4—304	5	—	5	—
SO_4—977,5 H_2SO_4—303,5	5	5	5	1

which initially had experimental silicosis, and animals which were free from silicosis. Five of the experimental animals which inhaled the combined oxide of sulfur and sulfuric acid aerosol died of experimental silicosis; 2 of these died three to four hours after the beginning of silicon dioxide inhalation. Another of the animals died at the end of 15 days inhalation, and the last animal died after 5 days of inhaling the combined sulfur containing pollutants. Only one of the disease - free animals died 2 and 1/2 days after experimental inhalation was discontinued.

Results of the investigation convincingly show that the animals under study were highly susceptible to chronic pulmonary deficiency elicited by the oxides of sulfur. However, in the absence of original data related to the lethal concentration of sulfurous gas to white rats, the present author was unable to properly evaluate the nature of the combined effect. This lack of pertinent data was supplemented to a degree by the following series of experiments conducted with guinea pigs.

Experiments with guinea pigs. Tests were conducted with 23 guinea pigs having experimental silicosis, and 21 free from the disease. The guinea pigs were divided into three groups and placed into experimental exposure chambers. Chamber No. 1 housed 7 guinea pigs having experimental silicosis, and 7 guinea pigs free from the disease; all were exposed to the inhalation of air containing 100 mg/m^3 of the sulfurous gas (average of 87.3 ± 15.4 mg/m^3). Chamber No. 2 contained 7 guinea pigs having experimental silicosis and 7 guinea pigs free from the disease and were exposed to the inhalation of air containg sulfuric acid aerosol in 10 mg/m^3 concentration with an average of 10 ± 2.4 mg/m^3; chamber No. 3 contained nine guinea pigs having experimental silicosis and 7 guinea pigs free from the disease. These were exposed to 118 hours inhalation of air containing sulfurous oxide vapor and sulfuric acid aerosol in similar concentration.

Concentrations were selected on the basis of following data: LD$_{50}$ for sulfurous oxide gas was at the level of 290 mg/m^3 regardless of age of guinea pigs. In this case death of the experimental animals occurred at later stages of continuous inhalation exposure. Accordingly, the concentration used in the experiment was 0.3 of the LD$_{50}$ for this sulfurous gas. The average sulfuric acid aerosol lethal concentration for guinea pigs weighing 1500 g was 50 mg/m^3, and for guinea pigs weighing 250 g it was 18 mg/m^3. It was determined by the interpolation method that LD$_{50}$ for guinea pigs weighing 646.4 ±50 g should be 29 mg/m^3 or the equivalent of 1/3 the concentration of the average lethal dose. Results presented in Table 2, indicate that no deaths occurred among guinea pigs which were free from silicosis or among animals which were free from pulmonary insufficiency. Inhalation of air containing sulfuric acid aerosol killed one of the guinea pigs, which had experimental silicosis, 36 hours after initiating the experimental inhalation.

Table 2

EFFECT OF SULFUR OXIDES ON GUINEA PIGS

Concentration in mg/m³	Number of animals		Number of animals	
	Silicosis positive	Number died	Animals tested	Animals died
SO_2—87,3	7	—	7	—
H_2SO_4—10,0	7	1+(1)[1]	7	—
SO_2—87,8 и H_2SO_4 10,5	9	5	7	—

(1) Parenthesis indicates one guinea pig which died on the 10th day after exposure termination. The author excludes it from the group of animals dead as the result of the poison administration of sulfur dioxide aerosols on the background of pulmonary insufficiency

It should be pointed out, in this connection that the animal under observation was a pregnant female which upon autopsy showed signs of nephrosis. Nevertheless, the fact that with a background of more profound pathologic processes sulfuric acid aerosols could cause death of the experimental animal even at concentration as low as 10 mg/m³, equivalent to 1/3 of LD_{50}, to healthy animals should be taken into consideration. The combined effect of sulfur oxides caused the death of 5 of the guinea pigs with experimental silicosis. One guinea pig died on the 19th hour of experimental inhalation, two - on the 32nd hour, one on the 40th hour, and one died on the 3rd day after experimental inhalation was discontinued. Results of the described investigation point convincingly to the fact that chronic pulmonary insufficiency lowered considerably the average concentrations of the tested sulfur oxides lethal to guinea pigs. The combined effect of different sulfur oxides enhanced their toxicity to guinea pigs.

The sum of concentrations expressed in parts of their average lethal values should be below unity as shown by the following example:

$$\frac{10\ \mathrm{mg/m^3}}{29\ \mathrm{mg/m^3}} + \frac{87.5\ \mathrm{mg/m^3}}{290\ \mathrm{mg/m^3}} = 0.34 + 0.3 = 0.64$$

It is suggested that this subject be reinvestigated on a more thorough basis using sick or otherwise susceptible experimental animals.

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HYGIENIC EFFECT OF CARBON BISULFIDE AND HYDROGEN SULFIDE SIMULTANEOUSLY PRESENT IN ATMOSPHERIC AIR (AN EXPERIMENTAL EVALUATION)

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The recently adopted maximal allowable concentration of atmospheric air pollutants in the USSR have been arrived at for the greater part on the basis of single pollutant components. It is now known that the presence of the air of two or more pollutant components may act in a way different from the action anticipated on the basis of individual components. The most frequent presence in the atmospheric air of two gaseous pollutants is that of carbon bisulfide and hydrogen sulfide. The presently enforced MAC for carbon bisulfide in atmospheric air was determined experimentally and recommended by R. S. Hildenskjol'd and Duan-Fun-Zhui. The problem assigned to the present author was to determine the hygienic effect of carbon bisulfide in atmospheric air in the presence of hydrogen sulfide. The effect of low concentration of the two poisonous gases on man was studied first by trying to establish **the odor perception threshold for odor**

sensitive persons, and the threshold of reflex effect on eye sensitivity to light, first of each component individually, and then of the two gases in combination.

Determination of the odor perception threshold was studied with the cooperation of 14 persons, 18 to 30 years of age, using the method recommended by the "Committee on Sanitary Protection of Atmospheric Air Purity" consisting of Prof. V. A. Ryazanov, K. A. Bushtueva, and Yu. B. Novikov. Results indicated that the hydrogen sulfide odor perception threshold was within the range of 0.014 and 0.03 mg/m^3, and of carbon bisulfide within the range of 0.08 and 0.5 mg/m^3, this was almost identical with the results previously obtained by Duan-Fun-Zhui and Hildenskjol'd. The study of odor perception threshold for combined carbon bisulfide and hydrogen sulfide indicated that 0.012 mg/m^3 was the lowest, or threshold odor perceptible concentration, for hydrogen sulfide, and 0.06 mg/m^3 for carbon bisulfide; the maximal non-perceptible odor concentration for each of the gases simultaneously present in the air was 0.010 mg/m^3 for hydrogen sulfide and 0.04 mg/m^3 for carbon bisulfide, as shown in Table 1.

Effect of low carbon bisulfide and hydrogen sulfide concentration on eye sensitivity to light reflexes was determined next, using adaptometer ADM and 3 test persons 18 to 28, years of age. Results are shown in Figure 1. The curves show that inhalation of hydrogen sulfide in 0.05 mg/m^3 and of carbon bisulfide in 0.010 mg/m^3 of carbon bisulfide and 0.012 mg/m^3 of hydrogen sulfide elicited statistically reliable deviations from the course of the original curves. Carbon bisulfide and hydrogen sulfide simultaneously present in the air in concentrations of 0.05 mg/m^3 and 0.010 mg/m^3 correspondingly elicited changes in eye sensitivity to light; these are concentrations which, as previously indicated were inactive insofar as reflex effect on eye sensitivity to light was concerned.

Table 1

DETERMINATION OF ODOR PERCEPTION THRESHOLD OF HYDROGEN SULFIDE AND CARBON BISULFIDE SIMULTANEOUSLY PRESENT IN THE AIR

Initials of testers	Concentration in mg/m^3					
	Nonperceptible individually		Perceptible in combination		Nonperceptible in combination	
	H_2S	CS_2	H_2S	CS_2	H_2S	CS_2
A	0,012	0,10	0,012	0,08	0,010	0,06
K	0,14	0,10	0,014	0,10	0,012	0,08
M	0,016	0,30	0,016	0,30	0,014	0,10
I	0,016	0,30	0,016	0,20	0,012	0,08
K	0,016	0,20	0,016	0,20	0,012	0,08
L	0,018	0,30	0,018	0,30	0,016	0,10
D	0,014	0,10	0,014	0,10	0,012	0,08
R	0,014	0,10	0,012	0,08	0,010	0,06
T	0,016	0,30	0,016	0,20	0,014	0,10
K	0,014	0,10	0,012	0,08	0,010	0,06
M	0,014	0,20	0,014	0,10	0,012	0,08
N	0,014	0,20	0,014	0,10	0,012	0,08
E	0,018	0,40	0,018	0,40	0,016	0,20
F	0,012	0,06	0,012	0,06	0,010	0,04

It is now known that the simultaneous presence of certain pollutants may effect the organism in a way of simple addition of similar effects, as potentiation of effects, the effect may be the result of antagonistic action.

In this connection, it is interesting to determine results obtained under such conditions related to odor perception threshold and threshold of reflex changes in eye sensitivity to light. It is known that the simple addition effect occurred when the sum of the active concentrations expressed in threshold fractions yielded a value closely approximating unity, and when the sum of inactive concentrations was less than unity. On the other hand, when the sum of active concentrations was less than unity, then one must assume that the effect may have been augmented or, as it is usually said, potentiated.

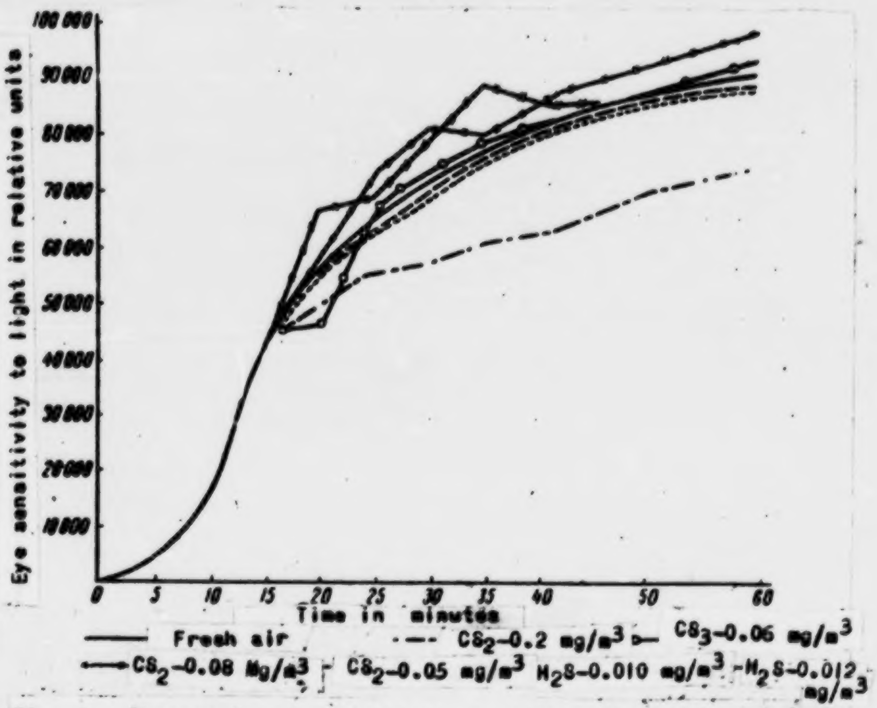

Fig. 1. Changes in eye sensitivity to light affected by inhalation of hydrogen sulfide and carbon bisulfide individually

In the present case of odor perception threshold determination the phenomenon is one of partial summation, as shown in Table 2. A similar relationship regularity prevails when the effect of two simultaneously present substances is determined with regard to the threshold reflex changes in eye sensitivity to light in terms of threshold values. For example, in the case of test person M the active summary concentration was 1.6, that is, in excess of unity, and the nonactive concentration sum was 0.99, or close to unity. Thus, it is seen that analysis of results obtained in determining threshold of odor perception and threshold of reflex changes in eye sensitivity to light, when H$_2$S and CS$_2$ were simultaneously present in the air, indicated that this was a case of partial summation of effects. A mixture of the two gases in their corresponding MAC had no effect on eye sensitivity to light.

Table 2

CONCENTRATIONS EXPRESSED IN FRACTION TERMS OF THRESHOLD ODOR PERCEPTION

Initials of testers	Total of H$_2$S and CS$_2$ threshold odor perception fractions in combination	
	Perceptible	Non-perceptible
A	1,15	0,92
K	1,12	0,95
M	1,55	0,95
I	1,46	0,82
K	1,46	0,82
L	2,35	0,78
D	1,07	0,85
R	1,15	0,92
T	1,50	0,95
K	1,15	0,92
M	1,16	0,95
M	1,16	0,95
E	1,40	0,93
L	1,50	0,99

The determination of maximal 24-hour average concentration was conducted on the basis of chronic experiments, using 45 white male rats weighing 110-150g; the rats were exposed to the inhalation of air containing the 2 gases in different concentrations. Rats were divided into 3 groups of 15 specimens each. Rats of group 1 were exposed to the inhalation of air containing 10 mg/m^3 of hydrogen sulfide, which is the equivalent of MAC for the gas in working premises. Rats of group 2 were subjected to the combined effect of carbon bisulfide in 0.01 mg/m^3 and hydrogen sulfide in 0.008 mg/m^3, both equivalent to average 24 hour concentration of these substances when present individually. Rats of group 3 served as controls. Experimental results were evaluated on the basis of observations regarding animals' general behavior, changes in the course of weight gain, changes in muscle antagonists chronaxy, blood cholinesterase activity, number of sulfhydryl groups in organs and in blood protein, also in blood protein fraction ratios and in total blood protein. At the end of the chronic experiment rats of the three groups were carefully studied pathomorphologically and pathohistologically.

Rats of the control group and 2nd group behaved normally and appeared well through the entire experimental period, gained weight at a normal rate, and showed no unusual deviations of any kind. Rats of group 3 were less active and showed a lower rate in weight gaining.

Effect of rats' exposure to the inhalation of air containing H_2S and CS_2 in combination was judged on the basis of indexes enumerated above. Results of observations indicated that statistically reliable shifts occurred in rats of group 1 in the muscle antagonists chronaxy beginning with the fourth week of exposure to the inhalation, as shown by curves in Figure 3. Data found in the literature regarding cholinesterase activity changes elicited by CS_2 and H_2S individually were used by the present author as a basis for evaluating results obtained in the present investigation. It was, thus, found that degree of cholinesterase activity depression in rats of group 1 beginning with the 5th week of inhalation exposure was statistically significant, as shown by curves in Figure 5. No changes in cholinesterase activity were noted in rats belonging to group 2.

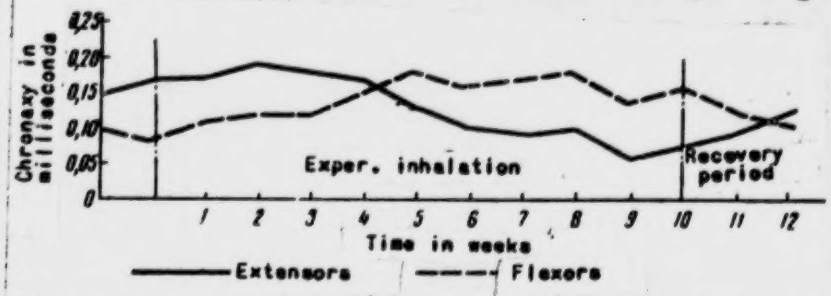

Fig. 3. Effect of carbon bisulfide alone on motor chronaxy of group Two rats in averages of five animals.

Present day physiology and bio-chemistry assigns an important role to the study of sulfhydryl compounds as related to normal and pathological processes of the animal organism. In this connection it was found that there existed a large group of toxic substances which bound free protein sulfhydryl groups. The present author also studied the concentration of SH-groups in the blood serum. Determinations were made by the iodiometric method. Blood was taken from the tail vein aseptically every twenty

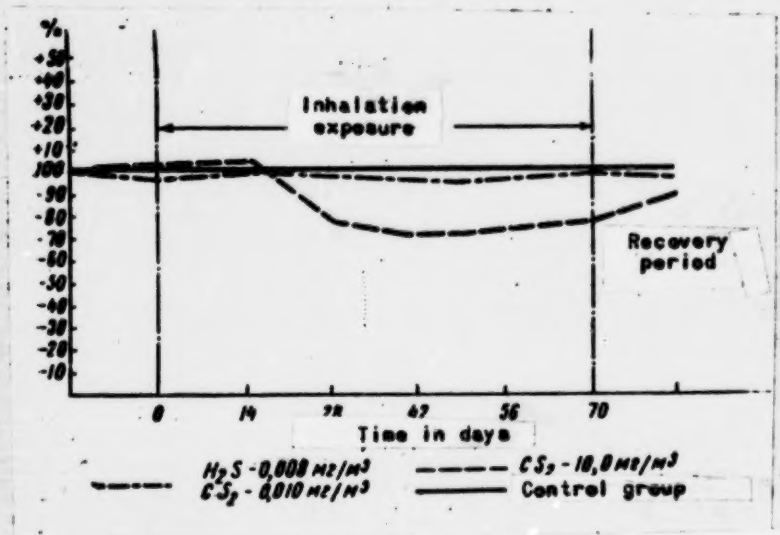

Fig. 5. Effect of carbon bisulfide alone and of hydrogen sulfide in combination with carbon bisulfide on whole blood cholinesterase activity of different rat groups

days. Statistically reliable and significant reductions were obtained in the total SH-groups values for the entire period of the experiment, which ranged from 19 to 25%; protein SH-groups were reduced by 14% as compared with the controls. No shifts in the number of total protein SH-groups were found in rats belonging to group 2. Total protein content in blood serum of rats belonging to groups 1 and 2 remained the same as in rats of the control group.

Autopsies perfor[illegible]n rats belonging to group 1, which inhaled air containing 10 mg/m^3 of CS_2 showed marked plethora of internal organs, especially of the lungs, liver, and kidneys. Lungs showed signs of congestive hyperemia accompanied by subplural hemorrhages. No gross organ pathology was noted in rats belonging to group 2. Histological studies of organ sections showed no notable changes in the axio-cylindrical processes of the neurons and of the sympathetic apparatus.

A study was then made of air polluted with H_2S and CS_2 simultaneously in the proximity of a synthetic fiber combine. Air samples were collected for the determination of maximal single concentrations of H_2S and CS_2 simultaneously using the aspiration method, at 500, 1500, 2000, 2500, 3500 and 4000 M on the lee side of the combine. Results indicated that the air was highly polluted with H_2S and CS_2, and that the CS_2 pollution exceeded the maximal single allowable concentration of this gas at 3000 M by 150 to 200 percent; at 3500 M the maximal allowable concentration was below the permissible level. H_2S concentrations were in excess of the MAC up to a distance of 2500 M from the combine.

Conclusions

1. Studies of CS_2 and H_2S threshold odor concentration for persons of high odor perception was 0.08 mg/m^3 and for CS_2 the concentration was 0.014 mg/m^3.

2. In simultaneous presence in the air of CS_2 and H_2S the result is one of partial odor intensity summation.

3. The combined effect of sulfur dioxide and hydrogen sulfide on eye sensitivity to light was also one of partial summation.

4. Chronic 24-hour daily exposure of rats to the inhalation of air containing 10 mg/m^3 of CS_2 elicited the following functional changes: it distorted the normal chronaxy ratio of the flexor and extensor muscles, changed the cholinesterase activity, effect the concentration of total and protein SH-groups. It also elicited pathological changes in some of the internal organs. Extent of all changes above referred to was verified statistically as significant.

5. Chronic exposure of white rats to the combined effects of CS_2 and H_2S in the air at concentrations equivalent to their corresponding MAC levels elicited no functional shifts in the animals' organism.

6. Results indicated that the previously adopted MAC for CS_2 and H_2S and the maximal single and 24-hour daily averages were below the threshold of notable summation effect; therefore, in evaluating atmospheric air polluted with CS_2 and H_2S simultaneously the standards adopted by the committee on sanitary protection of atmospheric air can be used without introducing any correction factors.

7. The study made of atmospheric air in the proximity of a combine producing synthetic fibers simultaneously polluted with CS_2 and H_2S suggests that official sanitary bodies should hasten with an introduction of effective methods for the purification of air surrounding such industrial plants.

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THE HYGIENIC EFFECT OF AVERAGE TWENTY-FOUR HOUR ALLOWABLE CONCENTRATIONS OF CHLORINE AND HYDROCHLORIDE GASES SIMULTANEOUSLY PRESENT IN ATMOSPHERIC AIR

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Experimental studies conducted in recent years by M. T. Takhirov, E. V. Elfimova, V. M. Styazhkin, and others, established allowable maximal single concentrations of chlorine and hydrochloride gases in atmospheric air at the levels of 0.1 and 0.05 mg/m^3 correspondingly under conditions of their individual or combined presence in the air. The present author studied the effect of chlorine and hydrochloride gas under chronic experimental conditions. Four groups of guinea pigs, 4 to 5 weeks old weighing 340 to 460 grams were exposed 24 hours daily for 3 months to the inhalation of air containing different chlorine and hydrochloride gas concentrations individually and in combination. Each group consisted of 8 guinea pigs. Animals of group 1 were exposed to the inhalation of 0.1 mg/m^3 of chlorine gas. Those of group 2 inhaled air containing 0.05 mg/m^3 of hydrochloride gas. Guinea pigs belonging to group 3 inhaled air containing 0.1 mg/m^3 of chlorine gas and 0.05 mg/m^3 of hydrochloride gas. Guinea pigs of group 4 served as controls. Procedures for maintaining constancy of the poisonous gases in the experimental exposure chambers were the same as previously described for similar experiments with other air pollutants. Tests indicated that concentration of chlorine gas through the experimental period averaged 0.12 ± mg/m^3, the hydrochloride gas averaged 0.07 ±0.001 mg/m^3. Averages of chlorine and hydrochloride gases simultaneously present in exposure chamber No. 3 averaged correspondingly 0.12 ±0.002 mg/m^3 and 0.06 ±0.001 mg/m^3.

Data found in the literature pertaining to the effect of simultaneously present chlorine and hydrochloride gases in inhaled air record only changes brought about by the combined toxic gases in the blood. However, data presented by different authors dealing with this subject were results of high gas concentrations and were strongly contradictory especially as regards morphological composition of the peripheral blood and the effect on bone marrow hemopoiesis. The present author studied this problem using low chlorine and hydrochloride gas concentrations.

Final experimental results were compared with results of preliminary observations which in the final study were given the scale value of 100. The hemoglobin level, number of erythrocytes and of reticulocytes were determined daily through the entire experimental period. Blood observations were made daily for the first 45 days and later only fortnightly. Bone marrow samples were taken once during the preliminary period and 3 times in the course of the entire experimental period. Data was processed statistically by the I. A. Oivin and by the M. P. Belen'kii methods. Inhalation of the two gases under investigation by the guinea pigs under the conditions of the experiment had no effect on the courses of the animals' weight gain their general state of health, or any other outward symptoms. Peripheral blood composition of the experimental guinea pigs showed a slight rise in the hemoglobin concentration within the limits of 2 to 6% physiological fluctuations. Number of erythrocytes persisted at the same level as in the control guinea pigs.

Hematocrit determinations made preliminary to the experiment and during the inhalation experiment showed no changes in the ratio percentages in plasma and morphological elements in the animals of all four groups. There was a slight increase in the hemoglobin concentration, probably caused by the erythrocytes increase, a phenomenon frequently observed in animals even under normal conditions. Likewise, no noteworthy fluctuations beyond the normal limits were noted in the experimental animals in the reticulocytes erythropoiesis. Neither were any significant changes noted in the leucocytic formula. Slight nuclear shifts to the right accompanied by a lymphopenia were noted in guinea pigs of groups 1 and 3 during the second and third weeks of experimental inhalation.

Chronic inhalation of hydrochloride gas by guinea pigs of group 2 had no significant effect on their percent ratio of the leucocytes in the blood. No other statistically verifiable or significant changes were noted in the other guinea pig indexes. The leucocytic formula showed a slight transient neutrophilic nuclear shift to the right. It is generally known that similar neutrofile changes are frequently observed at the early stages of effect on the organism of some extraneous factors. Lymphopenia occurring only in the presence of nuclear neutrophile shift to the right can be regarded not as an independent hemotological symptom, but as a phenomenon secondary to the neutrophilic replacement.

The present author was unable to record any substantial changes occurring in the bone marrow hemotophoresis induced by the effect of chlorine and hydrochloric gases, as shown by data listed in the unnumbered table.

Maturing of elements in the red propagulum of the bone marrow remained at its unchanged level, the hemopoiesis of red cells was free from any shifts. However, a comparison with average data of the control group indicated that some depression in erythropoiesis might have

CHANGES IN THE ERYTHROID BONE MARROW PROCESS IN GUINEA PIGS UNDERGOING CHRONIC EXPOSURE TO THE EFFECTS OF CHLORINE AND CHLOROHYDRATE EXPRESSED IN TERMS OF M ± m

Animal group and mg/3 of vapor in the air	Investigation period	Erthroblastogram						Maturity index	Leucoerythroblastic index	
			Erythroblasts			Normoblasts				
		Proerythroblasts	Basophilic	Polychromatophilic	Orthochromatic	Polychromatophilic	Orthochromatic			
Group one Chlorine 0.1	Pre-exptl.	1,0±0	2,0±0,27	5,0±0,4	1,0±0,34	9,0±0,43	2,0±0,27	0,9±0,04	4,4±0,20	t=2,5 p<0,05^{1}
	4th exptl. week	1,0±0	2,0±0	4,0±0	2,0±0	11,0±2,0	1,0±0	0,9±0	$\frac{4,3}{1}$ ±0,15	t=3,63 p<0,001
	8th exptl. week	1,0±0	2,0±0,41	6,0±0,64	1,0±0	11,0±1,04	2,0±0,36	0,9±0,02	$\frac{3,8}{1}$ ±0,19	
	12th exptl. week	1,0±0	2,0±0,27	5,0±0,43	2,0±0,41	8,0±0,32	1,0±0,32	0,9±0,03	$\frac{4,1}{1}$ ±0,31	
Second group, chlorohydrate 0.05	Pre exptl.	1,0±0	1,0±0,29	6,0±0,71	1,0±0	8,0±0,69	2,0±0,30	0,9±0,013	$\frac{4,6}{1}$ +0,31	t=2,5 p<0,05
	4th exptl. week	0	1,0±0,37	6,0±0,85	1,0±0	11,0±0,66	2,0±0,34	0,9+0	$\frac{4,0}{1}$ +0,18	
	8th exptl. week	0	2,0±0,37	5,0±0,41	2,0±0,32	12,0±1,5	2,0±0,71	0,9+0	$\frac{3,5}{1}$ +0,23	
	12th exptl. week	0	2,0±0,15	6,0±0,71	1,0±0	11,0±0,98	1,4±0,43	0,9±0	$\frac{4,0}{1}$ +0,30	
Third group Chlorine 0.1, Chlorohydrate 0.05	Pre-exptl.	1,0±0	2,0±0,33	4,0±0,52	1,0±0	9,0±0,72	2,0±0,21	0,9±0	$\frac{5,2}{1}$ ±0,34	t=3,8 p=0,002
	4th exptl. week	1,0±0	1,0±0,26	6,0±0,45	1,0±0	9,0±0,73	2,0±0,44	0,9±0,06	$\frac{4,3}{1}$ ±0,35	
	8th exptl. week	1,0±0	2,0±0,32	6,0±1,02	1,0±0,22	9,0±0,52	2,0±0,31	0,9±0,02	$\frac{4,3}{1}$ ±0,47	
	12th exptl. week.	1,0±0	2,0±0,23	5,0±0,58	1,0±0	8,0±0,62	2,0±0,30	0,9±0,01	$\frac{4,7}{1}$ ±0,32	t=3,61 p<0,01
Control group	Pre-exptl.	1,3±0,33	2,2±0,36	6,0±1,07	2,0±0,34	9,0±0.73	2,0±0,27	0,9±0,04	$\frac{3,7}{1}$ ±0,20	
	4th exptl. week	1,0±0	2,0±0,31	7,0±0,75	1,5±0,34	10,0±0,52	2,0±0,32	0,9±0,026	$\frac{3,5}{1}$ ±0,17	
	8th exptl. week	10,0±0	3,0±0,26	6,0±0,78	2,0±0,32	10,0±0,58	2,0±0,41	0.9±0,03	$\frac{3,3}{1}$ ±0,23	
	12th exptl. week	1,0±0	2,0±0,30	7,0±0,23	10,0±0,23	10,0±0,70	2,0±0,33	0,9±0,01	$\frac{3,4}{1}$ ±0,18	

appeared on the 4th week in guinea pigs exposed to the inhalation of air containing chlorine and hydrochloride gases simultaneously; this was paralled by an increase in the leucoerythroblastic index. On the other hand, this verificable statistical difference cannot be regarded as the result of chlorine-hydrochloride gas effect, especially since the level of original erythropoiesis in the control group differed considerably from that in the animals of the experimental group. Thus, it is seen that chronic exposure of guinea pigs to the inhalation of air containing simultaneously chlorine gas in 0.1 mg/m^3 and hydrochloride gas in 0.05 mg/m^3 concentrations had no substantial effect on the general condition, weight, blood composition, and bone marrow hemopoiesis of the experimental guinea pigs.

At the end of the experimental inhalation period all guinea pigs had been sacrificed and their tracheae,lungs, livers and spleens were examined parthohistologically; no changes suggestive of induced pathology were found in the organs of any of the experimental guinea pigs belonging either to the control or experimental groups. It is recommended that the previously adopted limits of allowable 0.03 mg/m^3 of chlorine gas and 0.015 mg/m^3 of hydrochloride gas should be retained without any revision.

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COMBINED HYGIENIC EFFECT ON THE ORGANISM OF CARBON BISULFIDE, HYDROGEN SULFIDE AND DINYL

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The combined hygienic actions of atmospheric pollutants had been studied first by K. A. Bushtueva in 1960-62 and pursued by V. M. Styazhkin in 1962 and by B. K. Baikov in 1963. Observations made by K. A. Bushtueva showed that the simultaneous presence in the air of sulfur oxides and sulfuric acid aerosol in concentrations of sub-threshold odor perception induced changes in optical chronaxy and elicited electrocortical conditioned reflexes. V. M. Styazhkin investigated the combined effect of chlorine and hydrochloride gases in their corresponding concentrations of threshold odor perception; they found that the effect was one of summation, and that when simultaneously present in their respective MAC the mixture failed to elicit any changes in the test animals; eye sensitivity to light and in the eye optical chronaxy. B. K. Baikov investigated the combined CS_2 and H_2S effect on the hygienic condition of atmospheric air. Determination of threshold odor perception of the two gases showed that in their simultaneous presence in the air they produced a total effect of partial summation, as shown by the fact that odor of $H_2S + CS_2$ was perceptible at 1.07 - 1.55 of their individual odor perception thresholds. The principle of partial summation also prevailed when the combined effect of the two gases was tested by the method of eye sensitivity to light. Chronic 24-hour experimental inhalation of air containing the two gases in concentrations equivalent to their corresponding MAC had no effect on the indexes used in this investigation.

Reports found in the literature described results of experiments conducted for the determination of effects of poison mixture present in the air in high concentrations under chronic experimental conditions. Results of two test series conducted by I. Ya. Shturm in 1938 for the determination of combined CS_2 and CO_2 action on the organism pointed to incomplete summation. Z. Kh. Filippova investigated the combined effect of CS_2 and some hydrocarbons, in 1961. Her experiments indicated that in the presence of hydrocarbons CS_2 toxicity increased to twice and three times the toxicity manifested when the pollutants were tested individually. The action of CS_2 in the presence of hydrocarbons in chronic experiments appeared as partial summation of the toxic effects. The task of the present investigator was to study the simultaneous hygienic effect of H_2S, CS_2 and of dinyl in concentrations equivalent to their corresponding MAC individually; also to make a survey of the hygienic state of stmospheric air in the vicinity of a plant producing synthetic cellulose fibers. CS_2 is the raw material used in the production of cellulose fibers, in the process of which 3-5% of the CS_2 vaporized into the atmospheric air. CS_2 in combination with other sulfur compounds was released during the intermediary reaction in the preparation of viscose fibers. Dinyl was used as the organic heat conveyor in the production of capronic fibers.

Most of the air polluting H_2S and CS_2 came from the viscose chemical and fiber spinning departments and from the CS_2 generating plant located on the same grounds. The CS_2 was removed from the spinning department by a centralized system via a discharge stack 120 m high, and from the chemical department by a system of exhaust ventilation installed 12-30 m above the ground. The plant's administrators estimated that tens of tons of CS_2 and H_2S gasses were discharged by the plant daily into the atmospheric air. The plant was not equipped with CS_2 and H_2S gas regaining installations. Dinyl, as an atmospheric air pollutant, came primarily from the chemical and spinning sections and from the boiler rooms, where dinyl was used as the calcining agent by circulating it through a closed system, from which it found its way into the atmospheric air as a result of leaky conduits.

Maximal single concentrations of hydrogen sulfide, carbon bisulfide and dinyl in the atmospheric air was determined in 730 samples collected during the spring-summer seasons of the year at 250 - 2000 meters on the lee side of the plant. Carbon bisulfide and hydrogen sulfide were determined by the methods recommended by the Committee on the Sanitary Protection of Atmospheric Air in 1959. Dinyl was determined by the Solomin modification of the Martynova method (1961). All samples collected at 250 meters were positive for carbon bisulfide, maximal concentration of which were 0.645 mg/m³. As the distance from the plant increased, maximal as well as average carbon bisulfide concentrations in the air decreased. However, carbon bisulfide

Table I

HYDROGEN SULFIDE AND CARBON BISULFIDE CONTENT IN THE AIR SURROUNDING THE PLANT PRODUCING SYNTHETIC FIBERS

Meters from the plant	Number of air samples		Concentration in mg/m³		No. of samples with indicated concn.			
	Total	Exceeding method sensitivity	Maximal	Average	Or less	0.009— —0.040	0.041— —0.080	0.081— —0.650
			Hydrogen sulfide					
250	41	41	0,645	0,253	—	3	—	38
500	32	19	0,220	0,169	—	2	2	15
1 000	62	45	0,132	0,067	1	10	19	15
1 500	65	43	0,210	0,055	1	26	9	7
2 000	60	27	0,110	0,041	—	20	3	4
		Carbon bisulfide			No of samples with indicated concn.			
					Or less	0.031— —0.150	0.151— —0.300	0.310— —0.652
250	52	52	0,652	0,188	3	24	17	8
500	35	30	0,435	0,149	6	15	4	5
1 000	62	40	0,200	0,082	11	26	3	—
1 500	65	34	0,108	0,042	12	22	—	—
2 000	60	24	0,109	0,035	15	9	—	—

concentrations in all samples collected at 2000 meters from the plant exceeded the 0.008 mg/m^3 official MAC. The maximal single carbon bisulfide concentration in samples collected at 2000 M also exceeded the adopted 0.03 mg/m^3 MAC by 300-400%.

Concentrations of dinyl found in the ambient air in the vicinity of the plant are listed in Table 2. Data in that Table shows that as the distance from the plant increased the concentrations of dinyl in atmospheric air decreased. For example, maximal single dinyl concentrations in samples collected at 1000 M from the plant were 5 times as high as the officially adopted MAC, and even at 1500 M it exceeded the adopted MAC by 200% in 42% of the samples. At 2000 M from the plant dinyl concentration in the air exceeded the 0.01 mg/m^3 official MAC in 10% of the tested samples. Thus, investigation showed that the atmospheric air in the vicinity was systematically polluted with a mixture of CS_2, H_2S and dinyl. Concentrations of each air polluting component frequently exceeded the corresponding MAC at 2 km from the plant. Therefore, it is recommended that the sanitary-protection zone for plants producing the type of artificial fibers under discussion should be raised from the presently stipulated 2 km to some greater appropriate width for plants which discharged into the atmospheric air 25 tons or more of CS_2 and H_2S per day.

Table 2

DINYL CONTENT IN ATMOSPHERIC AIR SURROUNDING THE PLANT PRODUCING SYNTHETIC FIBERS

Meters from Plant	No. of samples		Concn. in mg/m^3		Number of samples with indicated concns.			
	Total	No. above method sensitivity	Maximal	Average	0.01 and less	0.011—0.050	0.051—0.100	0.110—0.140
250	39	39	0,440	0,071	—	18	16	5
500	41	41	0,300	0,069	—	17	14	10
1 000	38	29	0,110	0,027	—	26	2	1
1 500	50	46	0,020	0,012	25	21	—	—
2 000	28	15	0,019	0,009	12	3	—	—

The toxic properties of dinyl have been inadequately investigated. According to Z. S. Kagan and E. V. Chechetkina dinyl is only slightly toxic. These authors reported in 1951 that brief inhalation of dinyl vapor presented no danger, but that prolonged inhalation caused headaches and irritation of mucous membranes. In 1955 Parmeggiani and Sassi examined workers employed in plants producing nylon. They found that employees complained of respiratory organ irritation, frequent headaches, loss of odor perception and of appetite. The author noted that at indoor 0.679 - 12.6 mg/m^3 dinyl concentrations the workers developed nasopharyngeal disturbances, conjunctival hyperemia, and liver enlargement was noted in some. No pathological changes had been noted in the blood picture. These authors concluded that the dinyl irritative effects to the organism were not cumulative.

In 1957, A. P. Martynova, reporting on her experiments with mice, stated that high dinyl concentrations in the air, such a 1 mg/li, inhaled for 2 hours elicited sharp morphological changes in the parenchymatous organs, in heart muscles, and in the lungs. Chronic inhalation of air containing 0.1 and 0.01 mg/li of dinyl four hours daily for six months elicited at the end of the experiment, reduced rate of weight gain, loss of vascular tonicity, and leucocytosis. Experimental animals suffered considerable loss in the nervous system's capacity to summate subthreshold impulses. These authors characterized dinyl as a resorptive poison, which elicited vascular tonicity and hemopoietic disturbances, and shifts in the functional state of the central nervous system. In 1961 G. I. Solomin studied the effect of low dinyl concentrations on the organisms' reflex activity. He showed that the threshold of dinyl odor perception in most odor sensitive persons was at the level of 0.06 mg/m^3. In 0.04 mg/m^3 concentration dinyl elicited reflex changes in eye sensitivity to light. Dinyl inhalation of air containing 0.03 mg/m^3 brought about conditioned electrocortical reflexes. In 0.01 mg/m^3 concentrations dinyl had no effect on the organisms' functional reactions.

G. I. Solomin's experimental data showed that inhalation of air containing 0.1 and 10 mg/m^3 of dinyl for 70 days (text does not indicate hours per day B.S.L) elicited in white rats some functional shifts such as lowered urinary coproporphyrin elimination rate, significant leucocytosis and lymphocytosis, changes in blood cholinesterase activity and in the muscle-antagonists motor chronaxy ratio. At 10 mg/m^3 dinyl concentration the above indicated changes appeared earlier and were more clearly expressed than at 0.1 mg/m^3. Inhalation of air containing 0.01 mg/m^3 of dinyl had no effect on the functional indexes of the experimental rats. In other words, 0.01 mg/m^3 of dinyl in the air was inactive; therefore, 0.001 mg/m^3 of dinyl in atmospheric air should be adopted as the maximal single and 24-hour average allowable concentrations.

The combined action of CS_2, H_2S and dinyl was studied next by determining the threshold odor perception concentration of each substance, and the effect of low concentrations of the combined substances. The functional state of rats' brain cortex as affected by the chronic action of the combined mixture was used as the indicator. The odor perception thresholds of the individual components were established by earlier investigators at 0.012 mg/m^3 for hydrogen sulfide (Duan-Fyn-Zhuii in 1959), 0.005 mg/m^3 for carbon bisulfide (R. S. Gil'denskjol'd in 1962), and 0.006 mg/m^3 for dinyl by B. I. Solomin in 1961.

The present author determined the odor perception thresholds of H_2S, CS_2 and dinyl combined using 19 test persons medically normal from the viewpoint of odor perception. The examination consisted of 243 determinations at different component mixture concentrations. Results listed in Table 3 are expressed in fractions of odor perception concentrations for each gas mixture ingredient. Only persons of high odor sensitivity were used

Table 3

CONCENTRATIONS OF HYDROGEN SULFIDE, CARBON BISULFIDE AND OF DINYL DETERMINED BY ODOR PERCEPTION WHEN SIMULTANEOUSLY PRESENT IN THE AIR

Concentration in mg/m^3			Total of odor perception fractions	Number of test persons	Number of persons	
H_2S	CS_2	Dinyl			Perceiving odor	Not perceiving
0,040	+0,010	+0,040	4,19	19	19	—
0,010	+0,010	+0,010	1,19	16	19	—
0,008	+0,005	+0,010	0,92	17	7	10
0,004	+0,004	+0,010	0,57	19	8	11
0,004	+0,004	+0,004	0,48	19	3	16
0,0035	+0,0035	+0,0035	0,43	19	—	19

in tests when the summary concentration, expressed in threshold fractions of each component gas amounted to 0.48 or higher. It has been known that the evaluation of mixture effect depended upon the value of a given indicator in the following order: If the summation index was greater than 2, the phenomenon was regarded as one of antagonism; an index value of less than 2 pointed to an independent action of the mixture components; where the index was greater than 1 but less than 2 the phenomenon was one of partial summation; where the index equalled 1 the phenomenon was one of complete or simple summation. An index of less than 1 pointed to potentiation. According to the above definitions the effect of carbon bisulfide, hydrogen sulfide and dinyl simultaneously present in the air was one of potentiation.

The reason for the present investigation was to check the rationality of existing maximal allowable concentrations for each indicated toxic gas. Accordingly, the data obtained, as shown in the preceding paragraphs, had to be evaluated on the basis of existing maximal allowable concentrations. The summation index for the lowest perceptible mixture was calculated as follows: $0.004/0.008 + 0.004/0.03 + 0.004/0.01 = 0.5 + 0.13 + 0.4 = 1.03$. The summation index for odor nonperceptible concentrations was as follows: $0.0035/0.008 + 0.0035/0.03 + 0.0035/0.01 = 0.43 + 0.11 + 0.35 = 0,99$. The mixture was odor nonperceptible when the sum of the ratios, as above indicated, was less than unity. Accordingly, based on data obtained for threshold odor perception concentrations the simultaneous presence of hydrogen sulfide, carbon bisulfide, and dinyl in atmospheric air in concentrations, the partial summation of which did not exceed unity, should be regarded as hygienically permissible. A. D. Semenenko demonstrated that inhalation of harmful substances in concentrations not odor perceptible affected the functional state of the central nervous system as determined by the method of electroencephalography. In 1934 Adrian showed for the first time that rhythmic flashes of light elicited synchronic adaptation to light flickering of a specific frequency.

N. N. Zislina in 1955, M. N. Danilova in 1960, and A. G. Kopylov in 1960 expressed the opinion that the rhythm adaptation method manifested the functional brain changes as a result of stimulation. Therefore, the present author used the functional encephalography method during simultaneous action of subthreshold odor perception concentrations in a mixture of three components. Tests were conducted with four practically normal persons 18-35 years of age. Electrical brain activity tests were made with the aid of an 8 - lead "Kaiser" encephalograph. Respiration encephalograms were made at the same time. Records were made of unipolar and bi-polar biocurrents leading from the occipital and temporal cortical sections. Light stimulation was induced by a Kaiser photo stimulator. Rhythmical light stimulation was induced at the rate of 8 per second, and at 0.1-0.6 joule intensity every other minute. Stimulation lasted 45 seconds in the course of which the intensity of the flash of lamp light changed nine times. The test person's eyes were closed while he was in a reclining position in the test chamber. The chamber air had been continuously ventilated. The source of light was kept at a constant distance from the observed person. A cylinder was placed in front of the test person equidistant between the eyes through which fresh air was flowing into the chambers at the rate of 25 li/min.

After double experimental control records had been made, the test persons were made to inhale for 5 min. air containing 0.0035 mg/m^3 of mixed carbon bisulfide, hydrogen sulfide, and dinyl, in concentrations which added up to a partial summation index of 0.99 for 5 minutes. On the 3rd inhalation minute a ten-minute electroencephalogram was recorded; and 10 seconds later the record was continued as described elsewhere in conducting similar tests.

Examination of the records had shown that values of summary amplitudes of adopted rhythms in test person V. was sufficiently above the amplitude manifested by the inhalation of fresh air; in test person M. the amplitude fell to a low statistically significant level. In other two test persons changes in the summary adaptation amplitude after inhalation of the gaseous mixture were not statistically significant. Thus, it appeared that the combined action of hydrogen sulfide, carbon bisulfide, and dinyl, in their corresponding subthreshold odor perception concentration. elicited changes in the summary amplitudes of the adopted rhythm in some of the persons.

Resorptive effect of the gas mixture was studied by the method of chronic experiments for 77 days; rats weighing 100-150 g were exposed to the inhalation of air containing the above specified mixture of the three gases under study. The study was made with 45 rats divided into three groups of 15 specimens each. Rats placed in chamber 1 inhaled air containing 0.01 mg/m^3 of each toxic gas component, which is the level of the average 24 hour allowable concentration of the mixture. Rats of group 2 were exposed to the inhalation of air containing the gas mixture of 3.5 mg/m^3 of each component. Rats were kept in 100 li inhalation chamber; the air was first passed through FVP-15 filter for preliminary purification and forced into the inhalation chambers at the rate of 20-25 li/min. Intermittent checks were made twice daily for the determination of the quantitative gas-air mixture stability. Test rats had been observed as previously described for a preliminary period of 30 days.

The control index series included dynamics of weight, muscle antagonists motor chronaxy ratio, blood cholinesterase activity, rate of coproporphyrin elimination via the urine, and morphological picture of the peripheral blood. Rats of group 1, which inhaled air containing 0.01 mg/m^3 of hydrogen sulfide, carbon bisulfide, and dinyl gas manifested statistically significant changes in the muscle antagonists chronaxy ratio beginning with the 35th day of experimental inhalation, as shown in curves of Figure 1. Beginning with the 30th day of the experimental inhalation this ratio showed substantial changes in rats of group 2. For example, the extensor and flexor chronaxy ratio exceeded unity in rats of this group on the 16th inhalation day. Thus, the simultaneous effect of carbon bisulfide, hydrogen sulfide, and dinyl under chronic conditions elicited the rats' functional nervous system changes expressed as lowered control by the central nervous system, as shown by muscle antagonists chronaxy ratios. Results presented in curves of Figure 2, show that blood cholinesterase activity in rats of the control group fluctuated within the limits of 37 - 43 min. with an average of 41.3 min. The acetyl choline hydrolysis time in rats of group 1 became considerably reduced indicating that activity of cholinesterase was enhanced. This was shown by the fact that acetyl choline hydrolysis time which was 40 min. at the initiation of the test, was reduced on the 45th day to 36 min. Rats belonging to group 2 showed statistically significant reduction in the cholinesterase activity time; this persisted through the entire experimental period. It has been established that intoxication of the organism with different poisons induced changes in the porphyrin metabolism. In this connection M. I. Gusev showed in 1960 that rate of coproporphyrin

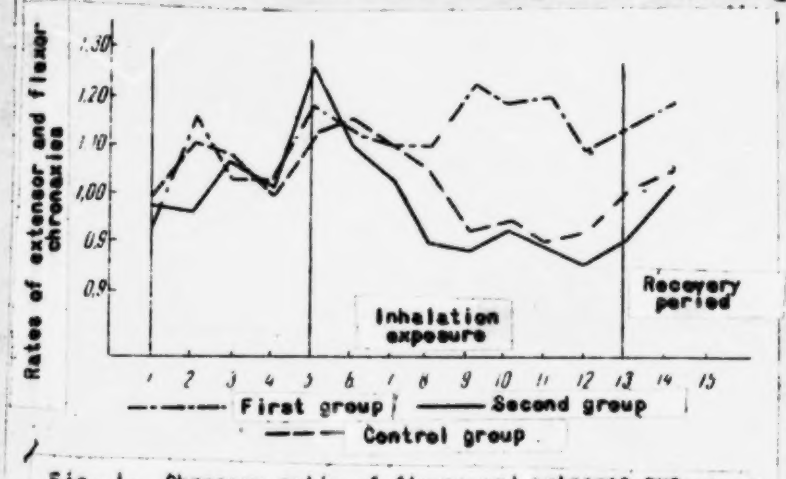

Fig. 1. Chronaxy ratio of flexer and extensor muscles in different animal groups.

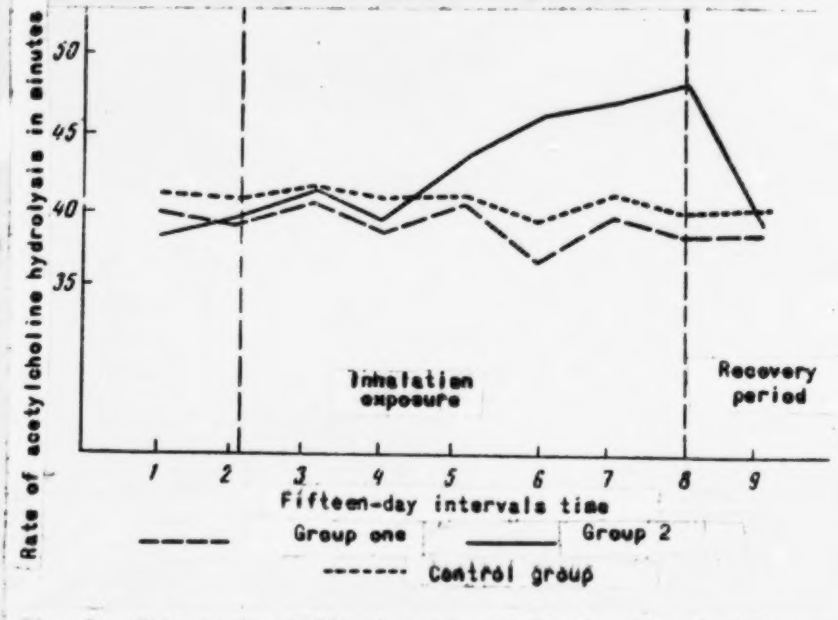

Fig. 2. Changes in cholinesterase activity in rats of different groups

elimination via the urine occurred in rabbits exposed to chronic inhalation of low concentrations, such as 10-11 μ/m^3 for 6 months.

The present author investigated the action of hydrogen sulfide carbon bisulfide, and dinyl mixture on porphyrin metabolism in experimental animals in chronic experiment. Observations were made at 10 day intervals. Results showed that no changes had been noted in porphyrin metabolism in rats of group 1. In rats of group 2 exposed to the experimental inhalation of air containing 3.5 mg/m^3 of the poisonous gas mixture, the amount of eliminated porphyrin followed the course of a wavy curve. Porphyrin elimination in rats of group 2 increased beginning with the 45th inhalation day, and rate of porphyrin elimination via the urine by rats belonging to the control group amounted to 40 - 50 μ per 100 g of rat body weight; in rats of group 2 the porphyrine elimination rate rose to 79 μ per 100 g of rat body weight. However, this increase in rate of porphyrin elimination at the end of the inhalation period fell to a level below that of the control group. Accordingly, no significant changes in the rate of porphyrin elimination had been noted.

Results of blood studies showed that a mixture of hydrogen sulfide, carbon bisulfide, and dinyl gases affected the morphological blood picture. The number of leucocytes in animals belonging to group 2, compared with those of the control group tended to increase never reaching noteworthy high levels. The leucocyte number in the blood of rats belonging to the control group ranged between 14,000 - 16,000 1mm^3; in rabbits of group 1 the range was 16,000 - 18,000, and in rats of group 2 it reached the level of 22,000, as shown in Figure 3. A moderate leucocytosis was noted consisting mostly of segmented nuclear leucocytes. Rats of group 2 showed a statistically significant increase in blood erythrocytes. At the end of the experimental inhalation period the number of erythrocytes rose to 11,000 per 1 mm^3; at the same time the number of such cells in the blood of rats belonging to the control group was 9,000,000.

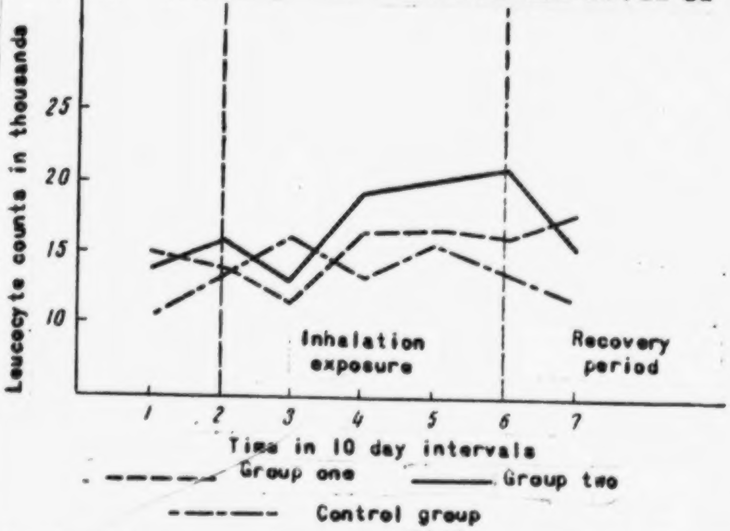

Fig. 3. Changes in blood leucocyte counts in rats of different groups

Accordingly, chronic inhalatinn of air containing a mixture of hydrogen sulfide, carbon bisulfide, and dinyl, each in concentrations of 3.5 mg/m^3, elicited in the experimental rats substantial changes in the muscle antagonists motor chronaxy ratio, in the morphological blood

composition, and in blood cholinesterase activity. Rats belonging to group 1, which inhaled air containing the three poisonous gases, each in 0.01 mg/m^3 concentration, which is above the 0.008 mg/m^3 MAC, showed no substantial changes in the morphologic blood pictures and in cholinesterase activity. However, ratio changes in the muscle antagonists chronaxy ratios were statistically significant. Accordingly, results of the study showed that the average 24-hour concentration of hydrogen sulfide, carbon bisulfide, and dinyl simultaneously present in inhaled air and expressed in fractions of the existing corresponding MACs required no revision.

Conclusions

1. Inhalation of air simultaneously containing hydrogen sulfide, carbon bisulfide, and dinyl lowered the threshold of its odor perception, thereby reflecting the nature of potentiation effect. A mixture of air polluting gases is odor nonperceptible if the sum of the quotients resulting from dividing each component's gas concentration by the respective maximal allowable concentrations was less than unity.

2. Chronic 24-hour inhalation by white rats of combined hydrogen sulfide, carbon bisulfide, and dinyl in 3.5 mg/m^3 of each component elicited changes in muscle-antagonists motor chronaxy, in blood cholinesterase activity, and in the morphological picture of the perpherial blood.

3. Prolonged inhalation of above mentioned gas mixture in 0.01 mg/m^3 concentration of each individual component had no effect on the mentioned physiological or systemic indexes, with the exception of muscle-antagonists motor chronaxy in which measurements of induced changes were verified statistically as significant.

4. The total average 24-hour concentration of hydrogen sulfide, carbon bisulfide, and dinyl, simultaneously present in the air, expressed in fractions of their respective maximal allowable concentrations should not exceed unity.

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DETERMINATION OF ATMOSPHERIC POLLUTANTS

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Determination of alcohols and organic acids in atmospheric and indoor air is still among the problems the solution of which present considerable difficulty. This applies in particular to the separate determination of alcohols and of carbon-containing acids. The present paper describes colorimetric methods for the determination of total alcohol, isopropyl alcohol paper, chromatographic partitioning of aliphatic alcohols, partitioning of aliphatic alcohols present simultaneously in the air, paper chromatographic partitioning of monobasic carbon containing acids of the fatty order C_1 - C_6 simultaneously present in the air, and determination of nitrobenze.

Colorimetric Determination of Aliphatic Alcohol

This method has been described by I. A. Selina in Gigiena Sanitariya 1962, No. 5, page 41. The principle of the method is based on the interaction taking place between the vanadium-oxiquinoline complex which resulted in the appearance of a rose color solution. Aldehyde, ketone and esters interfered with the reaction, if present in the sample, in amounts exceeding 1 mg. Sensitivity of the method is 0.005 mg in 2 ml.

Apparatus.

1. Aspirator or blow fan
2. Flowmeter of up to 5 li/min
3. Absorption apparatus, U-shaped, equipped with glass filter No. 1.
4. Flasks, volumetric, 50 ml capacity
5. Pipettes, 1 and 2 ml capacity, divided into 0.01 ml, and 5-10 ml capacity divided into 0.1 ml
6. Test tubes, colorimetric, equipped with ground-to-fit glass stoppers marked at 2 and 3 ml

7. Waterbath

8. Thermometer 100°

9. Rubber bulbs

Reagents

1. Standard aliphatic alcohol solution in xylol and benzene . Prepare initial standard solution from which prepare benzene solution containing 0.1 mg of the alcohol per 1 ml. Use aliphatic alcohol employed in the technological process of the plant in the vicinity of which the air investigation is to be conducted.

2. $CaCl_2$ dehydrated and redistilled benzene of 80° b. p.

3. Redistilled xylol of 138-144°

4. Aliphatic alcohols of series C_1 - C_{10}

5. Acetic acid, 5% solution

6. 2% of 8-oxyquinoline solution in 5% of acetic acid solution

7. Ammonium metavanadate. Prepare solution by using 0.08 g of ammonium vanadate in 100 ml of water. The solution will keep for one month

8. Vanadium oxyquinoline complex in benzene Prepare as follows: Use a 10 ml volumetric test tube equipped with a ground-to-fit stopper; place into it 1.2 ml of 5% acetic acid solution, 0.6 ml of 2% oxyquinoline solution, 2 ml of ammonium metavanadate, and 4 ml of benzene. Shake the tube for 2 - 3 minutes and leave stand until the precipitate settles to the bottom of the tube. The formed complex will be contained in the upper benzene layer. Decant it carefully and use as reagent for the determination of alcohols. This complex

should be freshly prepared before it is used.

9. Normal solution of sodium hydroxide

Collection of samples

For the determination of maximal single concentrations aspirate the air at the rate of 0.3 li/min. for 30-40 min., through 2 U-shaped absorption tubes equipped with No. 1 porous glass filter. Each absorber should contain 2.5 ml of benzene. Immerse the absorber in ice cold water. If absorption is made with the aid of solid sorbents the air should be aspirated through a glass tube 50 mm long and 8 mm inside diameter constricted at each end to 3-5 mm diameter. The tube must be filled with 1 g of activated charcoal and should have a pressure drop equivalent to 220-280 mm of water column. Insert cotton or fine wire plugs at each end of the tube to prevent the activated charcoal from falling out. Draw the air through at the rate of 2 li per minute. The time required for simple collection should vary with the alcohol concentration in the air.

Analytical procedure

The benzene contained in each of the two absorbers must be analyzed individually with the aid of analytical tubes 2 ml capacity equipped with ground-to-fit glass stoppers. Simultaneously prepare the standard scale as shown in Table 1. Place into all tubes of the scale and of the samples to be tested 0.35 ml of the above described complex, shake well and place into warm waterbath at 100° for 30 min. Then place into each tube 0.3 ml of normal sodium hydroxide solution and again shake vigorously. The xylol solution will acquire a pink color. Carefully decant the upper layers of the pink solution into dry colorimetric tubes and make determination by comparing color intensity of the sample containing and scale tubes. Where activated charcoal is used in collecting the air sample, the charcoal should be run from the aspiration tube into a test tube equipped with a ground-to-fit glass stopper followed by the introduction of 5 ml benzene. Thoroughly shake the tube containing the activated charcoal and place it for 60 min. into a waterbath heated to 56-60°,

Table I

STANDARD SCALE FOR ALCOHOL DETERMINATION

Test tube No.	0	1	2	3	4	5	6	7
Ml of standard solution	0	0,05	0,1	0,4	0,8	1,2	1,5	1,8
Ml of absorber solution	2	1,95	1,9	1,6	1,2	0,8	0,5	0,2
Mg of alcohol	0	0,005	0,01	0,04	0,08	0,12	0,15	0,18

then remove the glass-stoppered tube from the waterbath, shake vigorously, and leave rest until a turbid precipitate settles to the bottom; then take 1 ml of the clear benzene solution and analyze as above described.

Calculation

The volume of air taken for analysis adjusted to standard temperature and pressure was 112 li. Four-fifths of the sample was taken from each absorber for analysis. It was determined that the first absorber contained 0.005 mg of the substance, and that none was found in the second absorber. Accordingly, the total of xylol was (0.005 x 5) / 4 = 0.006 mg. Alcohol concentration in the air was (0.006 x 1000) . / 11.2 = 0.53 mg/m^3. In making determination of air samples collected by passing the air through activated charcoal use only 1/5 of the air sample.

Determination of Isopropyl or Secondary Alcohol

Principle of the Method

This method was described by I. A. Selina in Gigiena i. Sanitariya, 1962 No. 12. Isopropyl alcohol interacting with potassium persulfate yielded acetone. In alkaline medium the latter reacted with salicylic aldehyde and produced a yellow color which turned to bright orange as the concentration increased. Methyl, ethyl, butyl, isoamylic, hexalic alcohols and formaldehyde, acids and dinyl did not interfere with the reaction. Acetone interfered with the determination. The method sensitivity is 0.002 mg in 2 ml.

Apparatus

1. Aspirator
2. Absorber equipped with glass filter No. 1.
3. Colorimetric test tubes marked at 10 ml, equipped with ground-to-fit glass stoppers
4. Pipettes of 1, 2, 5, and 10 ml capacity
5. Volumetric flasks, 50 ml capacity
6. Waterbath
7. Thermometer, 100°

Reagents

1. Isopropyl alcohol

2. Standard aqueous solution of alcohol containing 0.1 mg of alcohol per 1 ml of the solution

3. Potassium persulfate ($K_2S_2O_8$), 1% solution

4. Potassium bisulfate, 5% solution

5. Sodium hydroxide 40% solution

6. Salicylic aldehyde, 20% solution in ethyl alcohol

Collection of Air Samples

For the determination of maximal single concentration aspirate the air at the rate of 0.3 li/min for 20-30 min., through U-shaped absorption tubes equipped with glass filter No. 1, containing 4 ml of water.

Analytical Procedure

Take 2 ml of the absorption solution from each aspirator, and place into each of 2 colorimetric test tubes. Simultaneously prepare the standard scale as shown in Table 2.

Table 2

STANDARD SCALE FOR ISOPROPYL ALCOHOL DETERMINATION

Test tube No.	0	1	2	3	4	5	6	7	8	9
Ml of standard solution	0	0,02	0,05	0,08	0,1	0,2	0,3	0,5	0,7	0,9
Ml of water	2,0	1,98	1,95	1,92	1,9	1,8	1,7	1,5	1,3	1,1
Ml of isopropyl alcohol	0	0,002	0,005	0,008	0,01	0,02	0,03	0,05	0,07	0,09

Into all test tubes of the scale and of the actual tests place 2 ml of $K_2S_2O_8$ 10% solution. Glass stopper as quickly as possible to avoid loss of the formed acetone and submerge into a 50-54° waterbath. Keep the test tubes in the waterbath at this temperature for 30 min., remove and cool to room temperature. Add to all tubes of the scale and test series 0.2 ml of 1% $K_2S_2O_8$ solution and without delay insert stoppers to prevent loss of forming acetone and place into a water bath, the temperature of which is gradually raised to 50 - 54° and leave stand for 30 minutes. Remove the tubes from the waterbath and allow to cool to room temperature. Add to all tubes of the scale and test series 0.2 ml of 5% bisulfite solution, mix well and add 2 ml of 40% alkaline solution. Then add to each tube of the scale and test series 0.2 ml of 20% of alcoholic salicilic aldehyde solution. Shake the individual tubes immediately after the reagent addition and place the scale and test sets into a waterbath heated to 80°, but not higher, for 15 - 20 minutes, cool, and compare colorimetrically.

Calculation of results

Air sample volume adjusted to standard temperature and pressure was 8 li, of which half was used in making the final determination. It was determined that the first absorber contained 0.008 mg and the second 0.002 mg of the isopropyl alcohol. Accordingly, isopropyl alcohol concentration in the air was: $(0.020 \times 1000) / 8 = 2.5\ \text{mg/m}^3$.

Paper Chromotographic Partitioning of Aliphatic Alcohols of Group C_1 - C_6 Simultaneously present in the Tested Air (Qualitative Determination)

Principle of the Method

This method was also developed by I. A. Selina. Paper chromatographic partitioning of aliphatic alcohols is based on their conversion into nonvolatile compounds, such as 3, 5, - dinitro-benzoates formed as the result of interaction between alcohol and 3, 5 - dinitrobenzoylchloride in the presence of pyridine. Partition the 3, 5 dinitrobenzoates paper chromotography, using dimethylformamide-hexane as the carrier. Place on the paper a part of the sample, and next to it an aproximately equal amount of a known mixture of aliphatic alcohols constituting the so-called reference spots. The heights to which the tested alcohol spots rise on the paper in relation to the height of the reference spots determine the type of the aliphatic alcohol. Alcohols of the higher series and other organic compounds did not interfere with the determination. The method sensitivity is $0.5\ \mu$ for each of the alcohols.

Apparatus

1. Aspirator or blow fan
2. Flow meter up to 5 li/min.
3. Absorbers equipped with porous glass filter No. 1
4. Colorimetric test tubes equipped with ground-to-fit glass stoppers 110 mm high and 11-12 mm in diameter marked at 3 ml
5. Separatory funnels, 100 ml capacity
6. U-shaped glass tubes 150 mm high, 5 mm in diameter
7. Chromotographic chambers

8. Volumetric 50 ml flasks
9. Micro pipettes of 0.1 - 0.2 ml
10. Enameled cruette 250 x 190 mm
11. Rubber bulbs
12. Electric heat ventilator
13. Glass pulverizers
14. Distillation flasks of 500 ml capacity
15. A modified Zaitsev absorber as illustrated in Limits of Allowable Atmospheric Air Pollution, 1962 volume 6, p. 201. (USSR Literature on Air Pollution and Related Occupational Diseases, Volume 9, p. 118, U. S. Department of Commerce O.T.S., Cat. No. TT-64-11574).

Reagents

1. Standard solutions of the alcohols in benzene, 1 ml of which contained 1 mg of the alcohol
2. Calcium chloride dehyrated redistilled, benzene, having a boiling point of 80°
3. Dimethylformamide redistilled and dehydrated of 153° boiling point
4. Hexane, CP dehydrated
5. Pyridine CP
6. Pyridine solution in benzene, 10%
7. 3, 5-dinitrol benzoylchloride. Dissolve 0.833 g of the substance in 50 ml of benzene. The solution will keep for 90 days
8. Tin chloride ($SnCl_2 \cdot 2H_2O$). Take 0.7 g

of $SnCl_2$ and place into a tube containing 5 ml of water; add 15 ml of HCl of 1.19 sp. gr. Add water to make 100 ml. This reagent will keep for 2 days only

9. Paradimethylaminobenzaldehyde
   Dissolve 1 g of this reagent in 95 ml of 95% ethyl alcohol; add 5 ml of hydrochloric acid 1.19 sp. gr. This reagent will keep for 2 weeks

10. Potassium hydroxide 50% solution

11. Hydrochloric acid 12% of 1.19 gr.

12. Acetone

13. Nitric acid, 1.39 - 1.40 sp. gr.

14. Silicagel, trade marked A.S.M. 0.5 mm granules. Heat the silicagel in 50% hydrochloric acid solution for 1 - 2 hours then place the silicagel into a separatory funnel and wash the granules to neutral reaction first with tap water and then with distilled water, then dry the silicagel at 100 ° and activate at 200-300° for 30 minutes

15. Chromatographic paper, trade marked M, of slow partitioning, manufacturered by the Leningrad Paper Plant

Collection of Air Samples

Where the air pollution is suspected to be high, samples may be collected directly by benzene absorption. In such case aspirate the air through 2 U-shaped absorbers equipped with porous glass filters No. 1. Each absorber should contain 2.5 ml of benzene. Rate of aspiration should be 0.3 li/min. for 20 - 30 minutes. Absorbers must be submerged in ice cold water. Where air pollution with the alcohol is light the air should be passed through the modified Zaitsev apparatus filled with 1 g of the silicagel at the rate of 5 li per minute for 40 - 50 minutes.

Analytical Procedures

Bring the silicagel, which now contains the tested substance in the absorbed condition, into the laboratory and transfer to U-shaped tubes in turn, connected to 2 U-shaped absorbers each containing 2 ml of benzene. Place the silicagel containing tube into a boiling waterbath and heat the air to 100°; aspirate through this system at the rate of 0.3 li/min. The aspiration is continued for one hour to assure complete desorption of the alcohol from the silicagel. Convert the alcohols into nonvolatile compounds as follows: place 1 ml of the standard solution, containing 1 mg of alcohol and 1 ml of the benzene, into a test tube equipped with a ground-to-fit glass stopper, add 1.5 ml of 3, 5-dinitrobenzoylchloride and 0.5 ml of pyridine solution in benzene. Mix and leave stand for 20 minutes, then pour the entire solution into a separatory funnel, rinse the test tube several times with benzene, and pour wash solution into the separatory funnel so that the funnel will now contain 5 ml of the solution. Add 5 ml of 50% potassium hydroxide. Shake vigorously and add 25 ml of water. Shake again and leave rest until the layers have clearly separated. Decant the water and wash the benzene solution with water once more by adding 25 ml of water. Now, add to the benzene solution 5 ml of 12% hydrochloric acid and shake vigorously. Leave stand until the mixture has separated into clearly defined layers. Decant the benzene layer, wash the tube twice with 5 ml aliquots of water.

The mixture constituting the, so-called, reference agent will keep at 4-6° for one to three months. Prepare the unknown alcohol mixture similarly. Place given quantities of the actual test solutions and of the reference solution on chromatographic paper as follows: use chromatographic paper measuring 14 x 50 cm and draw a standard pencil line 7 cm from the paper edge. Draw 3 pencil marks on this line 3.5 cm apart. Place some of the reference mixture on one of these pencil marks and some of the test solution on the other two pencil marks using a graduated micro pipette. Continue to add these solutions to the paper pencil marks until the spots measure 3 mm in diameter but not any more. Dry the spots by a warm air electric fan. Add enough of the reference mixture to the paper spots to make an alcohol deposition equivalent to 0.5 - 20 μ. The amount of reference alcohol to be taken depends upon its suspected concentration in the air. Where the concentrations are expected to be low deposit 0.5μ. Reduce the volume of test solution containing 3.5 dinitrobenzoate by evaporating the benzene to 1 - 2 ml at room temperature or by heating it to 60°. Then place 0.5 ml of the solution on the pencil marked spot. After the actual test and the reference solution have been placed on the paper, saturate it with 5% solution of dimethylformamide in acetone; prevent the dimethylformamide from coming in contact with the test and reference spots. Place the paper thus saturated between two light filter papers and air dry for 5 minutes. Saturate the paper with dimethylformamide as follows: First, saturate the edge of the paper to the starting line, then reverse the operation and continue the saturation from the starting line to the paper edge.

Proceed with the chromatographic partitioning in the descending order. First saturate the chamber with dimethylformamide vapor by suspending, in the chamber, a sheet of filter paper saturated with dimethylformamide. Other steps are identical with those usually employed in making chromatographic paper partitioning under identical conditions. After 10 to 12 hours the partitioning of the derivative alcohols should be complete. Yellow spots developed by the alcohol will remain in clear evidence for several months. Identify the unknown alcohols by the height to which the spots ascended with reference to spots of the known alcohols.

Chromatographic Paper Partitioning of Monobasic Carbon-Containing Acids of the Fatty Order Belonging to Group C_1 - C_2 Simultaneously Present in the Air (Qualitative Determination)

Principle of the Method

This method was developed by Yu. V. Dyuzheva. It is based on acid conversion into nonvolatile potassium derivatives of hydroxamic acids and partitioning it by descending paper chromatography. Esters and aldehydes interfere with the reaction. Reaction sensitivity is 5 μ.

Apparatus

1. Aspirator or blow fan
2. Flow meter up to 5 li per minute
3. Modified Zaitsev absorbers
4. Chromatographic chamber consisting of glass cylinder with ground-to-fit lid
5. Glass support and glass tray placed inside the chamber
6. Electrical heat ventilator
7. Pulverizer
8. Colorimetric 10 ml tubes
9. Porcelain dishes
10. 1, 2, 5 and 10 ml pipettes

11. 0.1 and 0.2 ml micropipettes

12. Round bottom flasks of 3 li capacity

13. Waterbath

14. Fifty ml volumetric flasks

15. Distillation flasks

Reagents

1. Standard fatty acid solutions of C_1 to C_6 series in methyl alcohol. Prepare by placing 10-15 ml of methanol in 50 ml volumetric flask; weigh and add 0.2 - 0.3 ml of the acid and again weigh. Add methanol to the 50 ml mark, shake well; use this as stock solution from which prepare solution containing 1 mg of the acid per 1 ml of the solution

2. Prepare absolute methanol as follows: Calcinate calcium oxide in a muffle furnace at 800 - 1000° for 1 to 2 hours. Use 300 g of calcium oxide per li of methanol. Place the mixture in a 3-4 li round bottom flask hermetically connected to a return condenser and distill all the alcohol as usual

3. Use silicagel ISM consisting of 0.5 mm granules. Treat the silicagel with 50% hydrochloric acid for 1 to 2 hours at warm temperature. Wash the silicagel to neutral reaction using lacmus paper as the indicator. Wash again with distilled water.

   Dry the silicagel at 100 - 105° and activiate at 300° for 20 - 30 minutes

4. Potassium hydroxide, 50% solution

5. Phenolphthalein 0.5% alcoholic solution

6. Alkaline hydroxylamine, dissolve 2.4 g of

hydroxylamine in 10 m. of boiling methanol, 2.8 g of potassium hydroxide dissolved in 10 ml of boiling methanol. Heat these solutions over a waterbath equipped with a return condenser. Cool both solutions to 40 - 50°, mix and add the alkaline to the hydroxylamine and cool in ice cold water. A rich percipitate of potassium chloride will be formed; remove it by filtration. Prepare the solution on the day of the determination

7. Ferric chloride, 1% solution in ethanol

8. Ethanol

9. Chromatographic paper of trade mark M, slow action, produced by the Leningrad Paper Factory

Sample Collection

Aspirate the air through 2 consecutively connected modified Zaitsev absorbers each containing 1.0 silica gel, at the rate of 5 li/min for 1 to 2 hours.

Analytical Procedure

Remove the silicagel from all absorbers; place into test tubes with ground-to-fit glass stoppers followed by adding 3 ml of ethanol. Continue extraction of the acids for 60 minutes with intermittent shaking of the tubes. Allow suspended material to settle to the bottom; take 1 ml of the clear solution from each of the absorbers and place into porcelain dishes; add one drop of phenolphthalein, and titrate with 30% solution of potassium hydroxide to a raspberry color; evaporate to dryness over a hot waterbath. Place the evaporated residue of potassium salts into a desiccator; simultaneously prepare calcium salts similarly from standard solutions of the acid. Do this by placing into procelain dishes 1 ml of standard formic acid, acetic acid, propionic acid, butylic acid, valerianic acid, and caproic acid, 1 ml of which contained 1 mg of the corresponding acid. Add to the porcelain dish one drop of phenolphthalein solution and titrate with 50% potassium hydroxide to the appearance of a raspberry color. Evaporate the liquid over a waterbath to complete dryness and place into a desiccator. Wash the dry residue of the test samples and of the standard scale into colorimetric tubes, using 1 ml of methanol for each porcelain dish. Add to the test tubes 1 drop of sulfuric acid of 1.82 sp.gr. Mix thoroughly and leave rest for 3 hours for the formation of esters. Three hours later add to each test tube 4 ml of alkaline hydroxylamine solution. Shake well and leave rest over night. The following day proceed with the paper chromatographic partitioning procedure. Place samples of the reference standard on the partitioning paper of the same make and quality as previously described. The paper should be 36 cm long and 12 cm wide. Draw a pencil line at 6 cm from the paper edge which will serve as indicated, the migration starting point. Two test samples and one reference standard can be entered on one paper 4 cm apart.

Deposit the reference mixture on the 1st pencil mark. Deposit test and reference solutions on appropriate pencil marks using 0.1 or 0.2 ml pipettes. Add standard solutions to their corresponding starting pencil mark in volumes of 0.025 up to 0.05 ml corresponding to 5 - 10 μ of the acid. Add test solutions to the paper in 0.1 - 0.2 ml. Dry the chromatographic paper rapidly by a stream of warm air in the chromatographic chamber. Take care to keep the spot diameters within the 2-3 mm. After the applied material has been dried, the chromatographic paper is partitioned by a carrier solution made up of a mixture of butanol, acetic acid, and and water in 4:1:5. ratios. Partitioning of the hydroxamic derivatives usually occurs within 18-20 hours, remove the chromatographic paper from the chamber and dry at room temperature. Develop the dried chromatogram in 1% solution of ferric chloride using a spray atomizer. The developed derivative acid spots should appear as violet spots on a yellowish background. Compare migration heights of test spots with migration elevations of reference spots, and identify the test spots chemically, thereby, determining the qualitative composition of the gaseous air pollutants.

Determination of Nitrobezene

Principle of the method

This method was developed and described by M. V. Alekseva, et al, in a book entitled "Determination of Harmful Substances in the Air of Production Premises". Moscow, 1954, p. 278. Convert the nitrobenzene into its dinitro form, which can be identified by the violet color. The reaction developed in the presence of methylethylketone, or acetone in alkaline medium. The method is not specific; benzene, toluol, chlorobenzene and acid interfere with the method. The method sensitivity is 0.25 μ in 2 ml.

Apparatus

1. Aspirator, or exhaust fan
2. Flowmeter, up to 2 li/min
3. Absorbers equipped with No. 1 porous glass filters

4. Colorimetric tubes equipped with ground-to-fit glass stoppers 110 mm high and 11-12 mm in diameter, marked at 2 and 3 ml

5. 50 ml volumetric flasks

6. Separatory funnels 50 and 75 ml

7. One and 2 ml pipettes divided into 0.01 ml and 5 and 10 ml divided into 0.1 ml

8. Waterbath

Reagents

1. Freshly redistilled nitrobenzene of 210.9° b.p.

2. Sulfuric acid 1.82-1.84 sp. gr.

3. Ammonium nitrate, desiccated at 80°

4. Nitration mixture prepared by dissolving 10 g of ammonium nitrate in 100 ml of sulfuric acid

5. Standard solution of nitrobenzene, prepared as follows: first prepare a stock solution by placing 10 - 15 ml of nitration solution into a 50 ml volumetric flask. Weigh the flask and content, then add 2-3 drops of nitrobenzene and weigh again. The difference between the two weights determines the amount of nitrobenzene. Mix the flask content and submerge into a boiling waterbath for 40 min. Remove and cool; add nitration solution to the 50 ml mark. Compute the amount of nitrobenzene contained in 1 ml. Place into another 50 ml volumetric flask a volume of the stock nitrobenzene solution containing 5 mg of nitrobenzene. Add nitration mixture to the 50 ml mark. One ml of the final mixture should contain 0.1 mg of nitrobenzene.

6. Methylethylketone - freshly prepared butanone, 70-80° M. P. colorless. Methylethylketone previously used can be used again, by first separating it from alkalies in a separatory funnel, dehydrating it over calcium chloride and double redistilling; the final colorless methylethylketone should remain color-free in alkaline medium
7. Sodium hydroxide, 40% solution
8. Freshly distilled alcohol, of 56° BP, in the absence of which methylethylketone can be used.
9. Ethyl ether of 35° BP

Collection of samples

For the determination of maximal single concentration of nitrobenzene aspirate the air on the lee side of its discharge into the air through two absorbers equipped with porous glass filter No. 1 for 20 min., at the rate of 1 li/min; each of the absorbers should contain 1 ml of the nitration mixture. For the determination of average 24-hour concentration aspirate the polluted air through two aspirators as above described for 2 hour period equally distributed over a continuous 24-hour day; or collect twelve 15-minute samples equally distributed over a continuous 24-hour day at the rate of 0.5 li/min.

Analytical procedure

Place the absorbers containing the air samples into a hot waterbath for 40 min.; cool. Pour the entire sample into a flask; rinse the absorbers with water several times using about 6-8 ml; add the rinse water gradually into the flask containing the combined sample avoiding overheating. Cool and neutralize with ammonia using lacmus paper as the indicator; pour into a separatory funnel, add 2.2 ml of butanone and shake for five minutes. Allow layers to separate and run out the lower layer of water through the funnel stem; decant upper layer through the upper funnel neck into a colorimetric tube; add butanone to the 2 ml mark. At the same time prepare the standard scale as follows: place 1 ml of the standard solution into a conical flask containing 8-10 ml of water; cool, neutralize with 25% ammonia solution using lacmus as the indicator and pour into a separatory funnel, to which add 10 ml of butanone and shake for 10 minutes; leave rest to allow clear separation into individual layers; remove lower water layer through the funnel stem by gradually openning the stopper; decant the upper butanone layer through the funnel neck into a test tube marked at 10 ml equipped with a ground-to-fit glass stopper. Add butanone to the 10 ml mark and shake lightly. The final standard solution should contain 10 μ of nitrobenzene per 1 ml. Use this standard solution for the preparation of the scale, as shown in Table 3.

Table 3

STANDARD SCALE FOR NITROBENZENE DETERMINATION

Test tube No.	0	1	2	3	4	5	6	7	8
Ml of standard butanone solution	0	0,025	0,05	0,1	0,2	0,4	0,6	0,8	1,0
Ml of butanone	2	1,975	1,95	1,9	1,8	1,6	1,4	1,2	1,0
Mkg of butanone	0	0,25	0,5	1,0	2,0	4,0	6,0	8,0	10,0

Add to all scale tubes and tubes containing the test material 0.5 ml of 40% alkaline solution; shake the racks holding the tubes for 15 minutes and make colorimetric determination.

Calculation

The aspirated air sample volume was 20 li and was the equivalent of 18.4 li of temperature and pressure adjusted air, the entire sample containing 0.5 μ of nitrobenzene was used in the analysis. Accordingly, the nitrobenzene concentration in the air was

$$(0.5 \times 1000) / 18.4 = 27.1 \mu \text{ or } 0.027 \text{ mg/m}^3$$

Where a photocolorimeter of spectrophotomer was available, the final determination can be made with the aid of calibrated curves constructed with the aid of data obtained on the basis of the scale shown above in Table 3.

STATISTICAL METHODS IN THE SEARCH OF A BASIS FOR ESTABLISHING MAXIMAL ALLOWABLE POLLUTANT CONCENTRATIONS IN ATMOSPHERIC AIR

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Use of standard methods in conducting basic experimental investigations has been universally recognized as a "scientific must". Statistical processing of experimental data, especially those obtained in the search of a basis for establishing maximal allowable pollutant concentrations in atmospheric air, is now regarded as of equal significance. Methods for statistical processing of general and clinical data have been described in many text books recently published by A. M. Merkov (1960), R. N. Biryukova (1962), A. Ya. Boyarskii (1955), and others. These have been widely used by different investigators under different conditions and for different reasons. The present communication in no way limits the use of methods described in statistical text books from being used in the field of air pollution investigations, especially in the phase which is concerned with the search of a basis for establishing MAC. The purpose of this communication is to stress the need of a standard universally adopted procedure of statistical data processing, which, in fact, was recommended by the "Committee on the Sanitary Protection of Atmospheric Air", affiliated with the USSR Government Sanitary Inspectorate.

Determination of a reliable degree of difference between compared value is a basic problem in statistical processing of experimental data, or of investigational results. In such instances it is essential first to compute: a) average arithmetic values; b) quadratic deviations; c) average errors of arithmetic averages of some indicator in the experiment and in the control before or after inhalation of a tested gas, etc. The arithmetic average (M) can be determined for groups of homogeneous combinations only, such, for instance, as arithmetic averages, of experimental animal weights in two groups under comparative study which are exposed to the effects of two different concentrations of a tested toxic substance under identical conditions.

$$M = \frac{\Sigma}{n}$$

Where v - denotes individual observation variants
Σ - is the summation sign
n - denotes the number of cases, or observations

However, the arithmetical average as such does not fully characterize or define the phenomenon under study; along with it the statistical analyst must know the degree of data dispersion and the statistical series variability measured by quadratic deviation designated by the Greek letter σ. Quadratic deviation can be determined by computing divergence from the average, or by some other less time consuming, but less precise, method of variation range. In the first procedure (see Table 1) the arithmetic average is determined first; this is followed by the determination of deviation (d) from the arithmetic average, then by the determination of quadratic deviation (d^2), and sum of quadratic deviation (Σd^2), and finally the average quadratic deviation ($d^2_{av} = \Sigma d^2 / n$), where the number of cases (n) is less than 30, then n-1 instead of n is taken as the denominator.

Quadratic deviation - sigma (σ) represents the square root of average quadratic deviation:

$$\sigma = \pm \sqrt{d^2_{av}} = \pm \sqrt{22.0} = \pm 4.69 \quad (2)$$

The other method for computing sigma according to range (amplitude) was described in detail by R. P. Biryukova in 1962. It makes possible rapid determination of quadratic deviation by the following formula:

$$\sigma = \frac{v_{max} - v_{min}}{K} \quad (3)$$

where v_{max} and v_{min} denote highest and lowest values of the studied series; K denotes the coefficient found in a special table which varied with the number of observations (cases) n.

Table 1

Gm of animal wt.	Deviation	Square of deviation from average
125	+4	16
115	—6	36
130	+9	81
118	—3	9
120	—1	1
123	+2	4
116	—5	25
118	—3	9
120	—1	1
125	+4	16

$$\Sigma v = 1210 \qquad \Sigma d^2 = 198$$

$$n = 10; \qquad M = \frac{\Sigma v}{n} = \frac{1210}{10} = 121;$$

$$d^2_{av} = \frac{\Sigma d^2}{n-1} - \frac{198}{9} = 22{,}0.$$

Table 2

MAGNITUDE OF K AT DIFFERENT OBSERVATION NUMBERS (n)

n	0	1	2	3	4	5	6	7	8	9
0	—	—	1,13	1,69	2,06	2,33	2,53	2,70	2,85	2,97
10	3,08	3,17	3,26	3,34	3,41	3,47	3,53	3,59	3,64	3,69
20	3,73	2,78	3,82	3,86	3,90	3,93	3,96	4,00	4,03	4,06
30	4,09	4,11	4,14	4,16	4,19	4,21	4,24	4,26	4,28	4,30
40	4,32	4,34	4,36	4,38	4,40	4,42	4,43	4,45	4,47	4,48
50	4,50	4,51	4,53	4,54	4,56	4,57	4,59	4,60	4,61	4,63
60	4,64	4,65	4,66	4,68	4,69	4,70	4,71	4,72	4,73	4,74
70	4,75	4,77	4,78	4,79	4,80	4,81	4,82	4,83	4,83	4,84
80	4,85	4,86	4,87	4,88	4,89	4,90	4,91	4,91	4,92	4,93
90	4,94	4,95	4,96	4,96	4,97	4,98	4,99	4,99	5,00	5,01

Following is an example of quadratic deviation determination by the range or amplitude method for a series identical with the one used in Table 1:

$$\sigma = \frac{v_{max} - v_{min}}{K} = \frac{130 - 115}{3.08} = 4.85$$

Table 2 shows that at n = 10, σ = 3.08.

The next step in statistical processing is computation of average error (m) of the arithmetic error, by using the following formula:

$$m = \frac{\sigma}{\sqrt{n}}$$

as stated above, when the number of cases or observations is less than 30

$$m = \frac{\sigma}{\sqrt{n - 1}} \quad (4)$$

For example, determination of error of arithmetical average of a statistical series shown in Table 1:

$$m = \frac{\sigma}{\sqrt{n-1}} = \frac{4,85}{\sqrt{10-1}} = \frac{4,85}{3} = 1,62.$$

Arithmetical average, quadratic deviation are basic parameters required for the determination of the reliability or significance of the difference between the experimental observation results and those of the controls. The indicated reliability is determined by way of computing the, so called, reliability coefficient symbolized by the letter t. This coefficient indicates the multiplicity factor (how many times) by which the indicator, in a given n of cases or observations must exceed its average error in order to be accepted as significant or reliable, and the results of statistical processing as sufficiently reliable. The reliability coefficient (t) is computed by the following formula:

$$t = \frac{M_1 - M_2}{\sqrt{m_1^2 - m_2^2}} \quad (5)$$

where M_1 and M_2 represent average errors of same indexes of juxtaposed groups.

The magnitude obtained for coefficient t is found in a table which presents three degrees of error probability, or of difference reliability at different n, as shown in Table 3 compiled by A. M. Merkov in 1960. Where the number (n) of observations (cases) is less than 30 n is computed with the aid of formula $n_1 + n_2 - 2$, in which n_1 is the number of observations in one of the juxtaposed groups, n_2 is the number of observations in the other group. Only that difference between two arithmetical averages can be regarded as reliable or significant in which the error probability (P) is less than 0.05 (5%).

Table 3

Number of observations	Value of coefficient t at probability error (P) not exceeding		
	0,05—5%	0,01=1%	0,001=0,1%
1	12,70	66,66	637,59
2	4,30	9,92	31,60
3	3,18	5,84	12,94
4	2,78	4,60	8,61
5	2,57	4,03	6,86
6	2,42	3,71	5,96
7	2,36	3,50	5,31
8	2,31	3,36	5,04
9	2,26	3,25	4,78
10	2,23	3,17	4,59
11	2,20	3,11	4,44
12	2,18	3,06	4,32
13	2,16	3,01	4,22
14	2,14	2,98	4,14
15	2,13.	2,95	4,07
16	2,12	2,90	4,02
17	2,11	2,92	3,96
18	2,10	2,88	3,92
19	2,09	2,86	3,88
20	2,09	2,84	3,85
21	2,08	2,83	3,82
22	2,07	2,82	3,79
23	2,07	2,81	3,77
24	2,06	2,80	3,75
25	2,06	2,79	3,73
26	2,06	2,78	3,71
27	2,05	2,77	3,69
28	2,05	2,76	3,67
29	2,04	2,76	3,66
30	2,04	2,75	3,64
	1,96	2,58	3,29

Many physiological tests can be used for the determination of the maximal single allowable concentration of atmospheric air pollution, such as threshold of odor perception, threshold of reflex action, etc. The statistical verification method or checking experimental changes effected by a given concentration of a tested substance, offers the investigator the possibility to obtain the concrete numerical characteristic of the phenomenon under investigation. Statements made in the preceding paragraphs can be illustrated by the following example: The problem is to determine changes produced in eye sensitivity to light by a given substance in 0.12, 0.6 and 0.3 mg/m^3 of amyl acetate in three test persons. Analysis of the total experimental material leads to the assumption that 0.3 and 0.6 mg/m^3 of the tested substance significantly affected the sensitivity to light; statistical processing of the experimental data verified the validity of such assumption. Checking procedure should be conducted as follows: Test the 0.3 mg/m^3 concentration using one of the three test persons for 3 days. Process results statistically by first computing the percent of enhanced eye sensitivity to light on the 20th, 25th, 30th, and 40th minutes as compared with the 15th minute. Run the gas into the test person's nares between the 15th and 20th minutes taken as 100%, as shown in Table 4. Record results in terms of relative (arbitrary) units (1/E).

Table 4

FEMALE Kh CONCENTRATION 0.3 MG/M^3

Time in minutes	1/II 1960 г.		2/II 1960 г.		3/II 1960 г.	
	I/E	Eye sensitivity to light in K at 15th min.	I E	Eye sensitivity to light in K at 15th min.	I E	Eye sensitivity to light in K at 15th min.
15	16 900	100	16 500	100	16 100	100
20	31 400	185,8	32 100	194,5	32 100	199,4
25	22 800	134,9	23 300	141,2	22 800	141,6
30	30 000	177,5	30 000	181,8	31 400	195,0
40	35 300	208,9	33 700	204,2	34 500	214,3

Data constituting eye sensitivity to light as recorded on the 20th minute of each of three test days make up the variation series:

185.8
194.5 Using the range method determine the
199.4 quadratic deviation (3)

$\Sigma = 579.7$
$M = 193.2$

$$\sigma = \frac{v_{max} - v_{min}}{K} = \frac{199.4-185.8}{1.69} = \frac{13.6}{1.69} = 8.1$$

Table 2 shows that where n = 3, K = 1.69 (original text shows K = 169). Now, compute the average error of arithmetical average m (4).

$$m = \frac{\sigma}{\sqrt{n-1}} = \frac{8.1}{\sqrt{2}} = \frac{8.1}{1.4} = 5.7$$

Results of the 25th, 30th, and 40th minutes of inhaling the same gas concentration and of fresh air (control group) are processed in a similar manner. Compare results yielded by the experimental amyl acetate inhalation in 0.3 mg/m^3 concentration with the results of the control inhalation by the same test persons on the 20th inhalation minute by computing coefficient t (5).

$$t = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}} = \frac{193.2 - 131.9}{\sqrt{2.82 + 5.72}} = \frac{61.3}{6.3} = 9.8$$

where $M_2 = 131.9$ (average eye sensitivity to light in percent on the 20th minute, as compared with same on the 15th minute of fresh air inhalation). $M_1 = 193.2$ (average eye sensitivity to light in percent on the 20th minute, as compared with the same on the 15th minute of inhaling 0.30 mg/m^3 of amyl acetate). $m_1 = 2.8$ (average error of average eye sensitivity to light in percent on the 20th minute, as compared with same on the 15th minute of fresh air inhalation). $m_2 = 5.7$ (ditto during inhaling 0.30 mg/m^3 of amyl acetate.

Data listed in Table 3 show that shifts manifested by test person who inhaled air containing 0.30 mg/m^3 of amyl acetate vapor on the 20th minute of the experiment, were statistically reliable, since $t > 6.86$ (see Table 3) when $n_1 = 3$, $n_2 = 4$ ($n = n_1 + n_2 - 2 = 5$), then reliability degree = 99.9%, and error reliability P did not exceed 0.001. Accordingly, it is possible to compute the statistical reliability or significance of experimental shifts with the aid of absolute or relative experimental values, and by comparing them with analogous control values. Similar computations can be made of values obtained in studies conducted for the determination of maximal single allowable pollutant concentrations in atmospheric air by other methods.

In studies conducted for the determination of a scientific basis for maximal allowable average 24-hour air pollutant concentrations experiments should be conducted on a chronic basis in a series of different properly selected concentrations of the air pollutant under study. Shifts taking place in the organism indicate effects of the different pollutant concentrations, and can be determined, or evaluated, by physiological, biochemical, morphological, and other tests.

In statistically processing the experimental material, constancy of the gas concentrations in the inhalation chamber is a factor of consideration. In this connection all results of analytical concentration determinations through the entire experimental inhalation period in each exposure chamber constitute a variational series. An example is presented in Table 5.

Accordingly, average chamber concentration was 0.29 ± 0.002 mg/m^3, and the average arithmetical error of the studied concentration was $m = \pm 0.002$, which is not a considerable magnitude. The series' dispersion characteristic can be computed with the aid of variation coefficient $V = (\sigma/M) \times 100$, i.e., percent which constituted quadratical deviation from the arithmetical average. In the example under consideration

$$V = (0.023/0.29) \times 100 = (2.3/0.29) = 7.9\%$$

In this case the quadratical deviation is of small value, amounting to 7.9%

of the arithmetical average. Such a dispersion of the studied concentration series can be regarded as a probable one. In the present example, effect of any given concentration on a given function of the organism, or on the entire organism, such as changes in body weight, will be evaluated first. Animal weight analysis can be conducted for two reasons. In the case of animals of identical initial weight it will determine the course of absolute weight changes induced by a given concentration of an investigated substance, as compared with the control; it will also serve as means of determining the reliability (degree of significance) of the weight changes. However, where experimental animals are picked at random, their initial weights may differ. Under such conditions the analysis should be applied to relative magnitudes expressed in terms of percent of the animal's initial weight. According to such a procedure the initial weight of each experimental animal is expressed in terms of 100%, and all calculations are conducted on the basis of pertinent relative values. An example is presented by data listed in Table 6.

Table 5

Concn. mg/m^3	Reoccurrence of concn. (symbol) during inhalation period (p)	$\nu \cdot p$	Deviation from average M(d)	$d \cdot p$	d^2p
0,35	3	1,05	+0,06	0,18	0,0108
0,33	9	2,97	+0,04	0,36	0,0144
0,31	24	7,44	+0,02	0,48	0,0096
0,30	58	17,40	+0,01	0,58	0,0058
0,28	45	12,60	−0,01	0,45	0,0045
0,27	22	5,94	−0,02	0,44	0,0088
0,25	14	3,50	−0,04	0,56	0,0224
0,23	5	1,15	−0,06	0,30	0,0180

$$n = 180$$

$$\Sigma \nu \cdot p = 52{,}05$$

$$M = \frac{\Sigma \nu \cdot p}{n} = \frac{52{,}05}{180} = 0{,}29$$

$$\sigma = \pm \sqrt{\frac{\Sigma d^2 \cdot p}{n}} = \pm \sqrt{\frac{0{,}0943}{180}} = \pm 0{,}023$$

$$m_{\text{cp}} = \frac{\sigma}{\pm \sqrt{n}} = \frac{0{,}023}{\pm \sqrt{180}} = \pm 0{,}002.$$

According to Formula (3) $\sigma = (130-108)/3.47 = (22/3.47) = 6.3$ According to Formula (4) $m = (6.3/14) = 6.3/3.7 = 1.7$ (This is the way it appears in the Russian Text, and no erratum is indicated, B.S.L). Compare with the control group in which $M = 128\%$, $m = 2.3$ and obtain $t = (128-120)/(\sqrt{1.7^2 + 2.3^2}\ ) = (8/2.86) = 2.8$. According to Table 3 at $n = n_1 + n_2 - 2 = 28$ the value of t (2.8) is greater than 2.76. Hence, the change in weight is significant, since its probability error (P) is 0.01 (1%).

Table 6

EXAMPLE OF COMPUTING STATISTICAL RELIABILITY OF BODY WEIGHT CHANGES IN RATS IN THE CHRONIC EXPERIMENT

Rats	1 III 1960		10 III 1960	
	Grams of absolute weight	% of wt. in re. to initial	Grams of absolute weight	Wt. in % of initial
1	90	100	105	117
2	85	100	103	121
3	97	100	112	115
4	100	100	108	108
5	82	100	99	121
6	80	100	104	130
7	97	100	115	118
8	93	100	114	123
9	88	100	106	120
10	91	100	113	124
11	100	100	113	113
12	84	100	102	121
13	89	100	106	119
14	95	100	118	124
15	98	100	120	123

$\Sigma = 1797$, $M = 120\%$

Note: There were 15 rats in the exposure chamber; rats were weighed every ten-day interval

Statistical processing of results yielded by the experimental study of porphyrin metabolism in animals chronically exposed to the inhalation of air containing poisonous gas, is conducted in a somewhat different manner. Generally, the body weight of the experimental animals differs, as does the 24-hour volume of collected urine and other physiological indexes. Therefore, it is more pertinent to compute the amount of porphirin eliminated daily per 100 g of animal body weight. Such a procedure makes possible to compare otherwise incomparable index values, such as μ of porphirin per 100 g of experimental animal body weight. In this connection it is well to remember that Li Shenj had shown in 1961 that the correlation coefficient between the amount of eliminated porphirin and body weight of rats in the course of experimental exposure to the inhalation of harmful substances was +0.90, which is, indeed, high. It must also be borne in mind that the total urine was collected from the rat group as an integral unit, therefore, it was not possible to build up a variational series for the computation of averages. In such instances the statistical processing should be conducted as follows: All porphirin determinations for each rat group should be divided into individual periods. For instance, 9 analyses in the course of the experimental inhalation period may be divided in the following periods: first month of experimental inhalation - 3 determinations; second month - 4 determinations; the recuperation period - 2 determinations (see Table 7.).

Compute average values for each experimental inhalation period, compare with the control, and compute the confidence coefficient t. Use t and n to compute the degree of reliability, if any, of porphirin elimination rate shifted from the rate of the control rat group. Data in Table 7 shows that in the course of the first month of chronic experimental inhalation the shifts were not statistically significant. During the 2nd month of the chronic experiment shifts in the porphirin elimination rates were statistically significant, since the corresponding values of the confidence coefficients t, 6.18 and 6.80, exceeded 5.96 at $n = n_1 + n_2 - 2 = 4 + 4 - 2 = 6.0$, which points to a high degree of reliability, with a probable error of less than 0.001. Data similarly derived during the recuperation period were found to be uncertain.

Table 7

RATE OF COPROPORPHYRIN ELIMINATED BY WHITE RATS EXPOSED TO INHALATION OF STYROL, ACCORDING TO LEE SHEIM

	Mkg of coproporphyrin eliminated per 100 g of animal body weight (symbol)										
	Group one			Group two			Group three			Control group (symbols)	
First month											
10/VI	1,31	1,47	1,05	0,85	1,13	1,74	0,90	1,39	1,0	1,46	1,90
20/VI	1,72			1,27			1,48			1,63	
30/VI	1,43			1,26			1,79			2,61	
Second month											
10/VII	0,40	0,58	6,18	0,56	0,61	6,80	1,24	1,43	1,18	1,39	1,63
20/VII	0,60			0,45			1,38			1,58	
30/VII	0,40			0,62			1,38			1,63	
9/VIII	0,90			0,79			1,70			1,93	
Recovery period рнод											
16/VIII	0,90	1,05	3,63	0,83	1,04	2,80	1,43	1,48	2,30	1,63	1,74
25/VIII	1,20			1,24			1,59			1,74	

Highly significant and important procedure of statistical data processing in evaluating shifts in motor chronaxy is the one concerned with the study of motor chronaxy ratios of the flexor and extensor muscles. Muscle antagonists chronaxy is controlled by the central nervous system; accordingly, any shift in the normal muscle antagonists chronaxy ratio points to a disturbance in the central nervous system. This emphasizes the important role played by statistical processing in the study of shifts in normal chronaxy ratios elicited by chronic experiments. For example, in 1963 R. Ubaidulaev studied the effect of furfurol on muscle antagonists chronaxy ratios of white rats. His data are presented in Table 8. The chronaxy ratio of flexor and extensor muscles in rats normally exceeded 1.1 -1.2. When the central nervous control over the chronaxy phenomenon becomes unfavorably affected, the flexor chronaxy becomes extended and exceeds the extensor chronaxy, causing the ratio coefficient to become less than unity. In this connection statistical processing of the results consists in comparing the latter, i.e., in determining the ratio coefficients. Arithmetical averages (M) of each group of rats obtained for chronaxy ratios in 5 rats are listed in Table 8.

Table 8

STATE OF EXTENSOR AND FLEXOR CHRONAXIES IN RATS CHRONICALLY EXPOSED TO THE INHALATION OF FURFUROL ACCORDING TO UBAYDULAEV

Period	Observation dates	Groups						
		First		Second		Third		Control
Before inhalation	17/X	1,11(0)	1,62	1,20(0)	2,33	1,18(0)	2,00	1,08
	25/X	1,16(0)	1,86	1,21(0)	1,14	1,17(0)	2,28	1,17
Inhalation period	31/X	1,08(0)	1,14	1,11(0)	0,33	1,11(0)	0,88	1,11
	8/XI	1,05(0)	0,37	1,12(0)	0,82	1,12(0)	0,48	1,09
	16/XI	0,92(*a*)	2,52	1,07(0)	1,33	1,1 (0)	1,71	1,11
	25/XI	0,81(0)	2,07	1,01(0)	1,86	1,09(0)	0,40	1,08
	2/XII	0,89(*a*)	2,90	0,97(*c*)	7,20	1,09(0)	0,88	1,11
	9/XII	0,76(*c*)	9,81	0,87(*c*)	5,23	1,08(0)	0,36	1,10
	17/XII	0,93(0)	3,66	0,93(*a*)	2,46	1,14(0)	0,72	1,11
	24/XII	0,83(*c*)	9,20	0,98(0)	2,00	1,15(0)	1,77	1,15
Recovery period	2/1	1,03(a)	2,50	1,11(0)	0,92	1,16(0)	1,33	1,15
	9/1	1,15(0)	0,40	1,14(0)	1,07	1,16(0)	0,96	1,15

Note: Reliability degree: a - 95%; b - 99%; c - 99.9%; 0 - Not reliable

Evaluate the confidence coefficient (T) at $n = n_1 + n_2 - 2 = 5 + 5 - 2 = 8$, and find that, as compared with the control group, rats of groups 1 and 2 manifested shifts during the second half of the experimental furfurol poisoning which were statistically of different probability degrees designated as follows: a - 0.05; b - 0.01; c - 0.001. Results obtained by other methods of study can be processed similarly in experiments designed for the experimental determination of maximal allowable concentrations (MAC) of atmospheric air pollutants.

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APPENDIX I
Approved and
inacted
Chief USSR Government Sanitary Inspector
T.A. Nikolaeva
20 June 1963, No. 442-63

MAXIMAL ALLOWABLE CONCENTRATIONS OF HARMFUL SUBSTANCES IN ATMOSPHERIC AIR OF INHABITED AREAS

№ n	Air pollutant	Formula	MAC in mg/m^3	
			Maximal single	Average 24 hr.
1	ACROLEIN	CH_2CHCHO	0,30	0,10
2	AMYLACETATE	$CH_3COOCH_2CH_2CH(CH_3)_2$	0,10	0,10
3	ANILINE	$C_6H_5NH_2$	0,05	0,03
4	ACETONE	CH_3COCH_3	0,35	0,35
5	ACETOPHENON	$C_6H_5-CO-CH_3$	0,003	0,003
6	BENZENE	C_6H_6	2,4	0,80
7	GASOLINE CRUDE OIL SULFUR POOR COMPUTED AS C		5,0	1,5
8	BUTYL ACETATE	$CH_3COOC_4H_9$	0,1	0,1
9	VINYL ACETATE	$CH_3COOCH==CH_2$	0.2	0,2
10	DICHLOETHANE	CH_2ClCH_2Cl	3,0	1,0
11	DIMETHYLFORMAMIDE	$HCO--N(CH_3)_2$	0,03	0,03
12	DINYL		0,01	0,01
13	METHANOL	CH_3OH	1,5	0,5
14	METHYL ACETATE	CH_3COOCH_3	0,07	0,07
15	METHYLMETACRYLATE	$CH_2:C(CH_3)COOCH_3$	0,1	0,1
16	MN AND ITS COMPOUNDS	Mn	0,03	0,01
17	AS INORGANIC COMPOUNDS HYDROGEN ARSENIDE EXCLUDED	As	—	0,003
18	CARBON MONOXIDE	CO	6,0	1,0
19	NITROGEN PENTOXIDE	N_2O_5	0,3	0,1
20	INERT DUST		0,5	0,15
21	METALLIC MERCURY	Hg	—	0,0003
22	SULFUR DIOXIDE (ANHYDRIDE)	SO_2	0,5	0,15
23	HYDROGEN SULFIDE	H_2S	0,008	0,008
24	CARBON DISULFIDE	CS_2	0,03	0,01
25	SOOTH (LAMPBLACK)		0,15	0,05
26	SULFURIC ACID	H_2SO_4	0,3	0,1
27	LEODPANODITSEXCEPT TETRAETHYL LEAD	Pb	—	0,0007
28	LEAD SULFIDE	PbS	—	0,0017
29	STYROL	$C_6H_5CH=CH_2$	0,003	0,003
30	FORMALDEHYDE	HCHO	0,035	0,012
31	TOLUILENEDIISOCYANATE	$CH_3C_6H_3(NCO)_2$	0,05	0,02
32	PHOSPHORIC ANHYDRIDE	P_2O_5	0,15	0,05
33	FLUORIDES		0,03	0,01
34	PHENOL	C_6H_5OH	0,01	0,01
35	FURFUROL	$C_5H_4O_2$	0,05	0,05
36	CHLORINE	Cl	0,10	0,03
37	HYDROGENCHLORIDE	HCl	0,05	0,015
38	CHLOROPRENE (2-CHLOROBUTADIEN-L.30	$Cl_2=CClCH==CH_2$	0,25	0,08
39	CHROMIUM HEXAVALENT COMPUTED AS CRO_3		0,0015	0,0015
40	ETHYL ACETATE	$CH_3COOC_2H_5$	0,1	0,1

Note 1. When atmospheric air contained SO_2 and sulfuric acid aerosol simultaneously, the sum of their respective concentrations computed according to the following formula must not exceed 1.

$X = (A/M_1) + (B/M_2) = 1$ or less than 1.

2. When atmospheric air contained H_2S, CS_2, and dinyl simultaneously, the sum of their respective concentrations computed according to the following formula must not exceed 1:

$X = (A/M_1) + (B/M_2) + (C/M_3)$, where X is the unknown,
(A/M_1), $B/M_2)$ and $C/M_3)$
are concentrations of the substance divided by their corresponding MAC when present in the air individually.

3. When atmospheric air contained H_2S and CS_2 simultaneously, then the MAC of each remains the same as if they were individually present in the air.

4. The list of MAC specified for harmful substances contained in atmospheric air in vicinities of inhabited areas issued and approved 14 February, 1961 as document No. 221-61 and reafirmed 13 April 1962, is hereby revoked and declared inactive.

Appendix 2,
approved by the decree of
the USSR Government
Committee for the Coordination of Scientific
Research.
Issued 25 July, 1963 as
Document No. 83

Temporary Method for the Computation of Dispersion in the Atmospheric Air of such Discharges as Ash, and Sulfur Containing Gases Released through Electric Station Smoke Stacks.

The temporary method for computing dispersion in the atmosphere of discharges emitted by electric station smoke stacks was developed and proposed by the A I. Voeikov Main Geophysical Observatory under the direction of Prof. M. E. Berlyand and his coworkers in collaboration with members of the Institute of Applied Geophysics and members of the F. F. Erisman Moscow Research Institute of Hygiene, and others. The following participated in the preparation of the text describing the method: Yu. Ya, Gerasimov, P. Ya. Tyurin, G. S. Zelivinskii,

M. S. Gol'dberg, N, Ya. Atarova, N. F. Dergachev, N. G. Zalogin, N. L. Oivin, V. S. Sorin, and V. V. Chuvachev.

Introduction. Until recently all USSR and foreign planning organizations resorted to the use of formulas for the determination of dispersion degree which were basically of an empirical nature applicable largely to sources of relatively low intensity located at relatively low altitudes. The formulas were not sufficiently general and could not apply correctly to different climatic conditions. Rectification of such a condition required the performance of special theoretical investigations and of practical experimentation under practical and natural conditions in the proximities of electrical heat and energy stations. Such studies were conducted in 1961-1962 in the region of the Shekinsk hydroelectric station, where an extensive complex of pertinent investigations had been organized and conducted by the members of the A. I. Voeikov Main Geophysical Laboratory, by the F. F. Erisman Moscow Research Hygiene Institute, by the F. E. Dzherzhinskii All-Union Heat and Energy Research Institute, and several other pertinent institutes. The previously developed theory of turbulent diffusion had been re-examined by modern methods for solving differential equations with the aid of electronic computers, and the results were checked and re-checked to a point of satisfactory agreement between results of computed and actual determinations. The proposed method for the calculation of smoke stack discharge dispersion is based on the elaborately developed theory and on the experimental investigations conducted in collaboration by the above mentioned institutes. The method, as described in the following paragraphs, should be regarded as a temporary expedient until further checking will establish its validity or superiority to to pre-existing methods.

The new computation method. 1. The degree of threatened ground air layer pollution by discharges emited by electric station smoke stacks is determined by the highest ground air layer pollution concentration C_M at a given distance from the smoke stack or stacks X_M under unfavorable meteorological conditions.

2. Under unfavorable meteorological conditions the value of maximal concentration C_M in mg/m^3 at level or slightly rugged terrain (1) and at even rate of harmful substances discharge coming from N smoke stacks of same heights can be computed by the following formula:

$$C_{\text{м}} = \frac{AMF_m}{H^2} \sqrt[3]{\frac{N}{V\Delta T}}, \qquad (1)$$

in which A - in sec$^{2/3}$ and degree$^{1/3}$ - is the coefficient which varied with the temperature of atmospheric stratification and which determined the conditions of vertical and horizontal dispersion of the pollution mixture in the air; M in g/sec denotes the total of harmful discharges coming from all smoke stacks; H denotes the smoke stack height in meters; V - denotes total volume of flue gases discharged through the smoke stacks in m^3 per second; ΔT in C° denotes the difference in temperature of gases emerging from the smoke stack (T_g) and the temperature of the atmospheric air (T_a); m and F denote dimensionless coefficients; m is a factor which accounts for the effect of flue gas emergence rate from the smoke stack mouth, W_0; F - is a factor which accounts for the effect of particulate matter settling out rate into the atmosphere. Formula (1) can be used for computing concentration of discharge admixtures coming from the smoke stacks at $H > 50$ m, $V/N > m^3/sec$ and $\Delta T > 30°$.

3. Coefficient A is applicable in unfavorable conditions when wind velocity reaches the dangerous value of $U^1{}_{м}$ which results in vertical inversion when the concentration at the ground air layer becomes maximal. For Kazakhstan, Central Asia and Central Siberia it can be assumed that $A = 200$; for the Northern and North-Western territories of USSR, the Ural and Ukraine assume $A = 160$. For Central European USSR and regions with similar climatic conditions $A = 120$.

4. Harmful admixtures discharged by electric station smoke stacks basically consist of SO_2, fly ash, and other dust. Calculation of such admixture concentrations as M in formula (1) takes into consideration discharges of sulfur dioxide (M_{SO_2}) and of ash (M_{ash}) after preliminary ash-catcher purification. Determination of M_{SO_2}, of M_{ash} and of V values are made on the basis of existing norms for boiler installation and heat calculations. Volume - V is determined by the amount of fuel consumed by a boiler of rated power generating, which varied with the diameter of smoke stack D (in meters) mouth and with the average velocity of flue gas emergence from the smoke stacks' mouth W_0 in m/sec

$$V = 0.25\, N\, \pi D^2\, W_0$$

In computing ΔT the value of T_w is determined approximately by finding ground surface, air temperature as shown in the climatological hand book for summer daylight hours. Coefficient m values at different W_0 m/sec are shown in the Table following:

W_0 м sec.	10—15	20—25	30—35
m	1,0	0,9	0,8

Note 1. Where local topography did not accord with indicated conditions ask the Chief Controlling USSR Hydrometeorological Service and the Chief USSR Government Sanitary Inspection for special directives.

Foot note 1. Dangerous velocity (rate) $U_{м}$ at the wind vane height, which is normally 10 m, can be determined by formula

$U_{м} = 065 \sqrt[3]{(V \Delta T/NH}$. For high power heat electrostations $U_{м} = 5 - 7$ m/sec.

5. In computing the concentrations of sulfur dioxide and other pollutants, the sedimentation of which is practically zero, the value of F must be assumed as unity ($F = 1$). In computing the concentration of fly-ash, which is a solid (particulate) pollutant the value of F must be assumed as 2 ($F=2$) when the average ash catching efficiency is not less than 90%, and $F = 2.5$ when the ash-catching efficiency was less than 90%.

6. Admixture concentration is at its maximum at distance X_m, expressed in terms of smoke stack height (H) and computed to an approximate degree by the following formula: $X_m = 20\ H$ (2).

7. Pollutant concentration $\underline{C}$ at hazardous (dangerous) velocity U_m at distance $\underline{X}$ from the smoke stack which is different from distance X_m can be determined with the aid of curve shown in Graph 1, where ratios of X/X_m are indicated along the abscissa and ratios of C/C_m are indicated along the ordinate; C_m can be computed with the aid of Formula (1) and X_m with the aid of Formula (2).

Fly ash concentration varies with the distance approximately as shown in Figure 1, when $X < 2\ X_m$; at higher $\underline{X}$, values diminished more rapidly, as shown by the dotted curve in same $\overline{\text{F}}$igure 2.

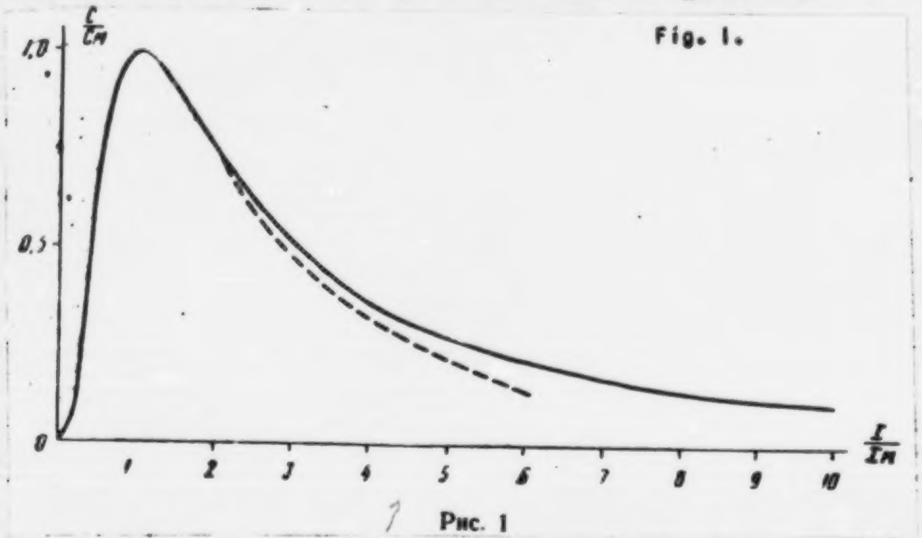

Fig. 1.

Рис. 1

8. For harmful pollutants entering the atmospheric air, their limiting concentrations are allowable depending upon their properties (Toxic) and duration of their action (effect) on the living organism. In accordance with sanitary standards for air of inhabited regions, the USSR Government Sanitary Inspectorate specifies, for single MAC values which reflected the basic characteristics of the air pollutant mixture harmfulness. Concentration of harmful pollutants, specially of SO_2 and of fly ash are determined under field conditions by collecting air samples for 20 min. In connection with this single MAC essentially constituted a 20 min. collection sample, the MAC value of 0.5 mg/m^3 has been established for SO_2 and for fly ash. The C_m concentration which is computed with the aid of formula (1)

also constituted a 20 min collection sample.

9. Discharge of the pollutant mixute M, height of smoke stacks, their number $\underline{N}$, and diameter $\underline{D}$, overheating ΔT, and rate of flue gases outflow W_0 must be selected so that C_s values did not exceed the MAC values; $C_s <$ M A C.

10. The maximal allowable smoke stack height can be determined by the following formula:

$$H = \sqrt{\frac{AMFm^3}{\text{ПДК}}} \sqrt[3]{\frac{N}{V\Delta T}}. \qquad (3)$$

If the discharge consisted of different harmful substances having different MAC values, then the smoke stack height is determined by the greatest $\underline{H}$ value obtained with the aid of formula (3) for each pollutant individually. Specifically, if $M_{SO_4} > FM_{ash}$ then H is determined on the basis of the SO_2 discharge, and when $M_{SO_4} < FM_{ash}$ the ash discharge should be used as the determining factor.

11. The maximal allowable discharge (MAD) in g/sec of harmful air pollutants at operating electrical stations can be determined by formula (4) at any given fuel consumption rate:

$$\text{ПДВ} = \frac{\text{ПДК} \cdot H^2}{AFm} \sqrt[3]{\frac{V\Delta T}{N}}. \qquad (4)$$

12. Maximal allowable fuel consumption D' in g/sec MAF at electric heat and power stations can be computed by formula (5):

$$\text{ПДТ} = H^3 \sqrt{\left(\frac{\text{ПДК}}{d_1 AFm}\right)^3 \frac{d\Delta T}{N}}, \qquad (5)$$

where d = v/D' - the amount of flue gases generated per unit of fuel weight, d = v/D' and di_1 = M/Di' denotes the amount of harmful discharge generated and emitted into the air per unit of fuel weight.

13. In the presence of highly unfavorable conditions, meteorological service data may indicate that an extensive temperature inversion prevailed over the smoke stacks giving rise to conditions favorable to the creation of high ground layer concentrations of harmful air pollutants threatening the health of nearby residents. Under such conditions authorities of the USSR Government Sanitary Inspection should require abatement of discharges into the atmosperic air.

14. In planning the enlargement of existing hydroelectric stations, or planning the construction of a new industrial production plant, the discharges of which are expected to contain harmful pollutant components similar to or identical with the ones discharged into the local atmospheric air by existing industrial enterprises, the background (or pre-existing) pollution concentration with such components must be taken into basic consideration. In such cases the background concentration $C_{bg} + C_s$ must not exceed the MAC. The smoke stack must be correspondingly higher in regions at atmospheric air of which had been previously polluted with

identical or similar industrial discharge components. According to formula (4) and without taking C_{bg} into consideration the smoke stack height must be H_0, whereas by taking the background factor into consideration the smoke stack height (H) in relation to H_0 will acquire the following expression:

$$H = H_0 \sqrt{\frac{MAC}{MAC - C_{bg}}}$$

15. Determination of background factor C_{bg} is the responsibility of the sanitary inspectorate bodies. It can be established by way of experimental determination of concentration $\underline{C}$ in the region where the new industrial construction is to arise. Such quantitative determinations should be conducted basically in regions located along a straight line connecting the area of the projected smoke stacks with the location of most extensive active pollution sources, and at times when wind direction is from the old pollution sources towards the new area of the projected industrial plants. If several industrial enterprises were operating along one such straight line (with a deviation not exceeding 1.0 - 1.5 H_0) then C_{bg} (background concentration) at the point of anticipated maximum will represent the summary concentration (C) of all such enterprizes. If the existing industrial enterprizes are not located along one straight line, then determination of (c) should be conducted at the point of assumed (c) maximum along each straight line running between the existing and projected industrial enterprizes. Under such conditions C_{bg} acquires the highest C value determined for each individual straight line. It must also be borne in mind that another sanitary hazard can arise as the result of adding another production plant to the existing system, a factor which will enhance the general pollution background to a point of exceeding the official MAC. In this connection, it is essential that consequent changes in maximal concentrations be evaluated in relation to other enterprizes by taking into consideration future supplemental concentrations from the projected plants under unfavorable wind conditions.

16. Computation of maximal concentration for electricity generating plants having smoke stacks of different heights and different discharge parameters should be conducted as follows: first determine C_m and X_m for each smoke stack and construct curves corresponding to changes in concentrations C and c and distance X, as shown in Figure 1; using the same system of coordinates construct such curves for each smoke stack. Then, add C values of all smoke stacks having identical X values; find the summary value of C_e in relation to distance X. The highest C_e value represents the value of C_m for the entire smoke stack system, and the corresponding X_m value represents the distance at which the maximum summary concentration can be found.

17. The following is an example for the computation of values representing maximal concentrations of harmful substances in ground sur-

face air layers established by the above described method and accepted active norms for the calculation of heat generation by boiler aggregates to be installed in a Ukrainian projected electric heat and power station. Admixture concentrations C at hazardous rate U_m at distances varying from X_m are determined for different X (1 - 2 km apart) on the basis of C_m and X_m computed as shown in curves of Figure 1.

Item No.	Denomination, designation, formula and calculation	Dimension	Magnitude
1	Electric station power output	M_{watt}	2400
2	Power output and number of units	M_{watt}	300x8
3	Type of fuel - anthracite, etc.		
4	Fuel heat capacity per working mass Qp_h	Ccal/kg	6010
5	Fuel ash content	%	16.7
6	Sulfur content - Sp	%	1.7
7	Excess of air upon emergence from flue (smoke pipe) - α	-	1.5
8	Loss of heat through incomplete mechanical combustion - q_h	%	3.0
9	Fuel ash portion carried off with gases via boiler aggregates gas flue - α_{car}	-	0.7
10	Efficiency coefficient of ash-catching installations (electrofilters) η_{ash}	%	98.0
11	T° of flue gases upon emergence from smoke stacks - T_s	C°	120.0
12	T° of atmospheric air - T_{air}	c°	25.0
13	Number of smoke stacks - N, computed on the basis of 1 smoke stack for 2 units of 300 M_{watt} output	-	4.0
14	Smoke stack diameter - D	m	7.0
15	Hourly fuel consumption for (8x300) 2400 M_{watt} output, determined on the basis of prevailing standards for the calculation of heat generated by the boiler aggregate (formulas 5-15)	t/hr	862.0
16	Volume of smoke stacks V_1 per 1 kg of fuel $V_s = V_{RO_2} + V^\circ_{N_2} + V_{H_2O} + (\alpha-1)V^\circ =$ $1.33 + 5.25 + 0.40 + (1.5 - 1.0) = 6.63$	nm^3/kg	10.3

Item No.	Denomination, designation, formula and calculation	Dimension	Magnitude
17	Total gas volume at exit from smoke stacks - $V = (V_2 x B x 10^3/3600)(120/273 + 1)$	m^3/sec	3540.0
18	Total SO_2 discharge from all smoke stacks - $M_{SO_2} + (Bx10^3/3600) x (Sp/100) x (\mu_{SO_2}/\mu_S) = +5.56xBxSp = 5.56x862x1.7$, where $\mu_{SO_2} = 64$ - mxw of sulfur	g/sec	8140
19	Total fly ash discharge - M_{ash} $M_{ash} = (Bx10^6/3600)(1-\eta_{ash}/100) x [(1-q_4/100) Ap/100\, \alpha_{yn} + Q_4/100] = 0.0278x B (100 - \eta_{ash})(0.679 Ap+q_4) = 0.0278x862(100 - 98)(0.679x16.7+3)$	g/sec	687.0
20	Coefficient accounting for effect of pollutants rate of precipitation from the air - F:		
	a) for SO_2 - F	-	1.0
	b) for fly ash - F'	-	2.0
21	Rate of flue gas emergence from smoke stack mouth - W_0 $W_0 = (4V/\pi D^2 N) = 4x3540/3.14x7^2x4$	m/sec	23.0
22	Coefficient m	-	0.9
23	$\Delta T = T_\Gamma - T_B = 120 - 25$	C	95.0
24	Coefficient A	$sec^{2/3}\,{}^{\circ}C^{1/3}$	160.0
25	In the present case $M_{SO_2} > FM_{ash}$ therefore value of H is determined from SO_2 discharge. Preliminary H value on the basis of $M_{SO_2} = 8140$ g/sec. $H = \sqrt{AM_{SO_2} x F_2/MAC}\,\sqrt[3]{N/V\Delta T} = \sqrt{160 x 8140 x 1 x 0.9/0.5}\,\sqrt[3]{4/3540x95}$	m	224
26	Highest approximate smoke stack used for electric stations, H = 250 m; for this reason same value was assumed in this case, - smoke stack height -H	m	250.00

Item No.	Denomination, designation, formula and calculation	Dimension	Magnitude
27	Maximal discharge concentration SO_2 - Cm_{so_2} $C_{m SO_2} = AM_{so_2} \times F_m / H^2 \sqrt[3]{N/V\Delta T}$ $= (160 \times 8140 \times 1 \times 0.9/250^2)$ $\sqrt[3]{4/3540 \times 95}$	mg/m^3	0.43
28	Maximal fly ash discharge $C_{m ash} = AM_{ash} \times F_m / H^2$ $\sqrt[3]{N/V\Delta T} = 160 \times 687 \times 2 \times 0.9/250^2$ $\sqrt[3]{4/3540 \times 95}$	mg/m^3	0.07
29	Dangerous wind velocity at wind vane $U_m = 0.65 \sqrt[3]{V\Delta T/NH} = 0.65$ $\sqrt[3]{3540 \times 95/4 \times 250}$	m/sec	4.5
30	Distance at which maximal discharge volume occurs $X_m = 20 \times H = 20 \times 250$	m	5000

For $SO_2 C_m$ 0.43 mg/m^3, $X_m = 5$ km

At x = 2, 4, 6 . . . km x/X_m = 0.4; 0.8; 1.2 . . .

Find in Figure 1 that c/C_m are equal correspondingly to 0.48; 0.96; 0.98 . .

Then at	x = 2 km	c =0.21 mg/m^3	
	x = 4 km	c =0.41 mg/m^3	
	x = 6 km	c =0.42 mg/m^3	Figure 2

With the aid of obtained data, curve in Figure 2 was constructed with C_{so^2} as the function. A similar curve can be constructed with C_{ash} in relation to x.

Fig. 2 -

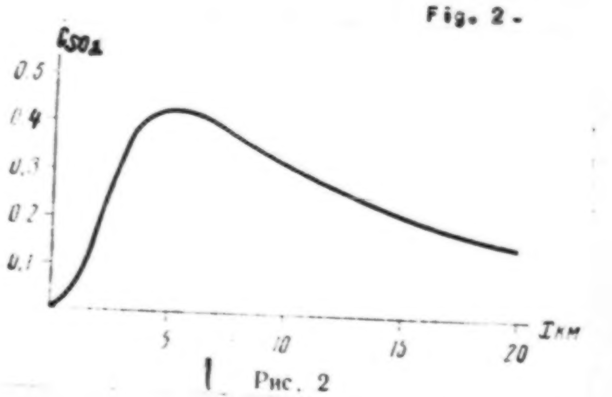

Рис. 2